

CHAPTER II

LITERATURE REVIEWS

Natural rubber

Natural rubber (NR) latex is a milky fluid obtained from the *Hevea brasiliensis* tree (Figure 1), which is a major agricultural product of Thailand. NR latex composed of *cis*-1,4-polyisoprene is widely used for manufacture of tires, gloves, condom [1]. The raw NR latex typically contains 33% rubber in the form of particles with a modal diameter of 400 nm [10]. The hydrophobic rubber hydrocarbon centers are surrounded by a half unit membrane and associated proteins, including the rubber elongation factor that was presumably involved along with cytoplasmic prenyl transferase in the synthesis of the rubber in the particle [11]. The remaining 67% of latex is the rest of the cytoplasm including nuclei, mitochondria, and various other organelles including the lutoids-specialized lysosome-like vacuoles [12]. The tendency of latex to coagulate is due to the effect of citrate and phosphatases released from the fragile lutoids neutralizing the negative charge on the rubber particles, allowing them to adhere to each other. The NR latex contains hevein and hevamine in the lutoids, the former a chitin-binding protein with antifungal activity [13], the latter a chitinase with lysozyme activity which may also be antifungal [14].

Most of the latex is processed at the site of production [15, 16] by coagulating the colloidal rubber with acetic or formic acid, and making it into sheets, crepe, blocks or granules which are then manufactured into rubber goods such as tires, shoe soles and tubing. This process eliminates most of the cytoplasmic proteins before manufacture. About 10% of NR latex is kept in the colloidal form, with ammonia added as an anticoagulant/preservative and the rubber particles were concentrated by centrifugation to approximate 60% dry rubber content. The latex concentrate thus contains rubber particles suspended in ammoniated cytoplasm (often with additional preservatives such as tetramethylthiuram disulphide) and is kept in this form for at least 3-4 weeks to mature before manufacture into gloves, condoms, catheters, elastic bands, foam or other latex goods [17]. During manufacture, various chemicals may be

added such as vulcanizers (4,4'-dithiomorpholine), accelerators (e.g. tetramethylthiuram disulphide, mercaptomixtures, benz-thiazoles.), stabilizers and antioxidants (thiocarbamates and paraphenylene diamine) some of which may be the cause of contact (type IV) sensitization.



Figure 1 Rubber (*hevea brasiliensis*) tree [18]

The protein content of latex is responsible for the majority of generalized allergic reactions to latex. The proteins are found in three distinct formulations: water-soluble, starch-bound or latex-bound. There are at least 240 potentially allergenic proteins in the processed latex product. Eleven sensitizing proteins have been identified or cloned so far and have been assigned allergen designations of Hev b1-b11 by the International Union of Immunological Societies [19].

The protein content of latex gloves can vary up to 1,000-fold among different lots marketed by the same manufacturer and 3,000-fold between gloves from different manufacturers [20]. In general, the protein content and allergen levels are highest in powdered examination gloves and lowest in powderless gloves, which undergo

additional washing and chlorination. These processes result in protein denaturation and decrease the total protein content.

Clinical manifestations of latex allergy [19]

Three distinct types of reactions can occur in persons exposed to latex containing products: irritant contact dermatitis, Type IV hypersensitivity or Type I hypersensitivity (Table 1).

Table 1 Types of reactions to latex gloves

Reaction	Signs/Symptoms	Cause	Management
Irritant Dermatitis	Scaling, drying, cracking of skin	Direct skin irritation by gloves, powder, soaps	Identify reaction, avoid irritant, possible use of glove liner, use of alternative product
Type IV - Delayed Hypersensitivity	Itching, blistering, crusting (delayed 6-72 hours)	Chemical additives used in manufacturing (such as accelerators)	Identify offending chemical, possible use of alternative product without chemical additive, possible use of glove liner
Type I - Immediate Hypersensitivity		Proteins found in latex	Identify reaction. Avoid latex containing products.
A. Localized contact	Itching, hives in area of contact with latex		Use of non-latex or powder-free,

Table 1 (Cont.)

Reaction	Signs/Symptoms	Cause	Management
B. Urticaria	(immediate)		low-protein gloves
C. Generalized reaction	Runny nose, swollen eyes, generalized rash or hives, bronchospasm, anaphylaxis		Anaphylaxis protocol

Irritant dermatitis

Irritant dermatitis is the most frequently observed reaction to latex products, accounting for 80 percent of work-related reactions to latex gloves [21]. This type of reaction results from the drying action of the corn starch and/or other irritant chemicals found in gloves and can be exacerbated by the soaps and mechanical irritation required for surgical scrubbing. Irritant dermatitis is not mediated by the immune system and is not a true allergy. However, the resulting deterioration in skin integrity possibly enhances absorption of latex protein allergens and is believed to accelerate the onset of allergic reactions.

Type IV delayed hypersensitivity

Type IV delayed hypersensitivity, also called T-cell mediated contact dermatitis, allergic contact dermatitis, and delayed hypersensitivity, directly involves the immune system, in contrast to irritant dermatitis. Among the immunologic responses to latex, 84 percent are Type IV [22]. This type of reaction is usually a response to the chemical additives used during the manufacturing process, specifically the accelerators, rather than to the latex proteins themselves. The resulting skin reactions are similar to those caused by poison ivy. Like poison ivy, the skin rash usually appears 6 to 72 hours after initial contact and may progress from a mild

dermatitis to oozing skin blisters. It is important to recognize that not all patients with Type IV reactions progress to Type I reactions. However, 79 percent of Type I patients previously had Type IV symptoms [23].

Type I immediate hypersensitivity

Type I immediate hypersensitivity, also called IgE mediated anaphylactic reaction or anaphylactic reaction, results when an antigen induces the production of an antibody of the immunoglobulin E class. Re-exposure to the inciting antigen triggers a cascade of events, including the release of histamine, arachidonic acid, leukotrienes and prostaglandins. Reactions usually begin within minutes of exposure. Symptoms can run the entire spectrum from mild (skin redness, hives, itching), to more severe (cough, hoarse voice, chest tightness, runny nose, itchy or swollen eyes), to life-threatening (bronchospasm and shock). Type I reactions from latex exposure have been reported in patients and health care workers and in a variety of clinical settings, including: vaginal deliveries [24], gynecological examinations [25], dental procedures [26], intra-abdominal [27] and genitourinary [28] surgery and during the act of donning gloves.

Protein assay techniques [29]

This review considers four commonly used methods: the biuret method, Coomassie Blue (CB) G-250 dye-binding, the bicinchoninic acid (BCA) assay and the Lowry method for the colorimetric determination of protein concentration in solution.

Biuret method

The biuret reaction is based on the complex formation of cupric ions with proteins [30]. In this reaction, whereby peptides containing three or more amino acid residues form a colored chelate complex with cupric ions (Cu^{2+}) in an alkaline environment containing sodium potassium tartrate. This became known as the biuret reaction because it is chemically similar a complex that forms with the organic compound biuret ($\text{NH}_2\text{-CO-NH-CO-NH}_2$) and the cupric ion. Biuret, a product of excess urea and heat, reacts with copper to form a light blue tetradentate complex. In addition, polypeptides have a structure similar to biuret, they are able to complex with copper by the biuret reaction as shown in Figure 2. The biuret reaction is the basis for a simple and rapid colorimetric reagent of the same name for quantitatively

determining total protein concentration. The working range for the biuret assay is 5-160 mg mL⁻¹, which is adequate for some types of industrial applications but not relatively sensitive enough for most protein research requirements [31].

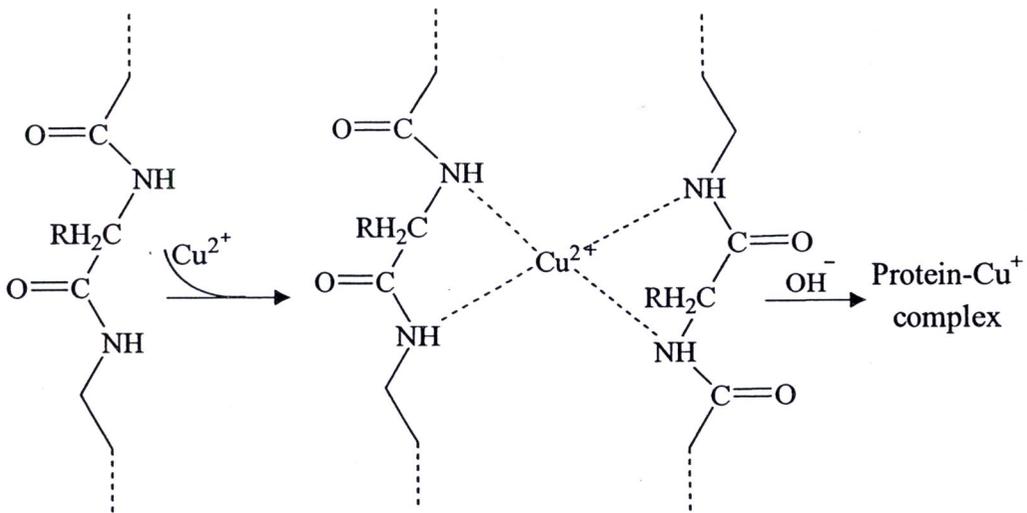


Figure 2 Diagram of the biuret reaction between protein and cupric ion [31]

Coomassie Blue (CB) dye-binding assay (Bradford assay)

The use of the metachromatic response observed on the binding of CB to proteins for the determination of protein concentration was popularized by Bradford [32]. Fazekas and coworkers [33] presented a systematic analysis of the use of CB R-250 and Procion Brilliant Blue RS to estimate protein concentration on electrophoretic strips. Both of these dyes were originally developed for the textile industry. CB is a triphenylmethane dye belonging to the magenta family. A good correlation was obtained for the value obtained with a micro-Kjeldahl procedure and CB for albumin and rabbit gamma-globulin; a difference was observed with lysozyme (0.75 compared with 1.16 obtained with the Kjeldahl procedure). Reisner and coworker [34] extended these observations by the use of CB G-250 in perchloric acid to stain gels. The effective use of CB for the determination of protein concentration in solution required the work of Bradford [32]. The ease and high sensitivity of the CB protein assay have driven its extensive use for the determination of protein concentration in a wide variety of protein samples. The exact chemical interactions or binding properties of CB G-250 dye are illustrated in Figure 3. The dye binds to proteins using three types

of interactions. The primary interaction of the dye with proteins occurs through arginine, a basic amino acid, which interacts with the negatively charged sulfate groups through electrostatic interactions. Other weaker dye-protein interactions include the interaction of the aromatic rings of CB G-250 dye with the aromatic rings of amino acids, such as tryptophan, through electron stacking interactions. Finally, the dye also weakly interacts with polar amino acids that have hydrophobic R-groups, such as the aromatic ring of tyrosine. The binding of the protein to the dye converts the dye to a stable, unprotonated, blue form. The intensity of the blue color indicates the level of protein in a sample. The more intense the blue color, the more protein present in the sample [35].

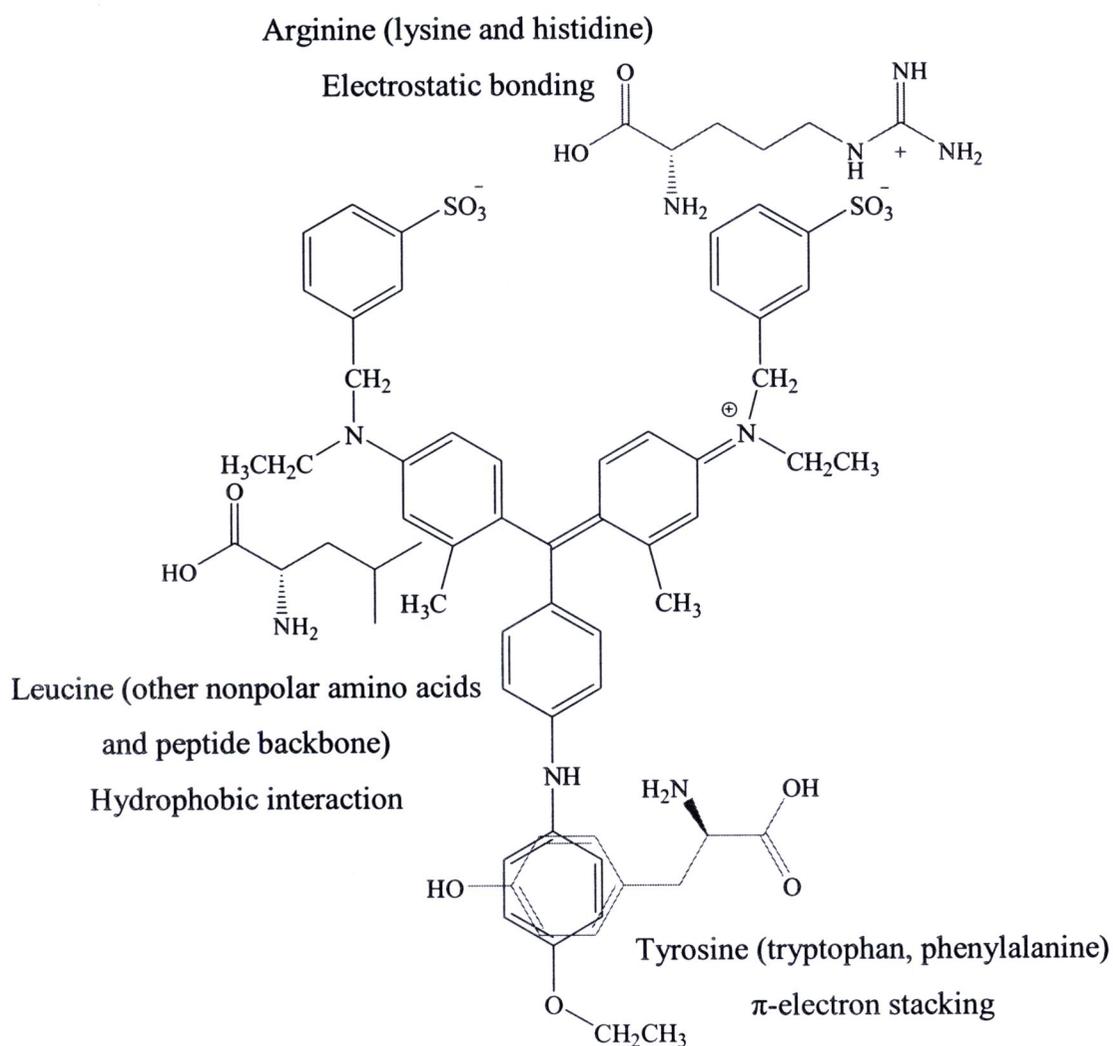


Figure 3 Coomassie blue G-250 interactions with amino acids [35]

Bicinchoninic acid (BCA) assay

The Lowry assay using BCA was developed by Smith and coworker [36]. This assay uses BCA to detect the cuprous ions generated from cupric ions by reaction with protein under alkaline condition. This method is sensitive and relatively easy to perform but still is markedly influenced by protein-to-protein variation. In the original study [36], Smith and coworker observed a variation in maximal color development as a function of protein composition similar to that observed with the Lowry reaction with the exception of avidin. The variation as a function of protein composition could be decreased by reaction at 60°C. The studies which the BCA reaction was used to measure salivary protein concentration, values obtained with the BCA reaction were equal to those observed with the Lowry reaction (1.08 mg mL⁻¹) but half to those measured with the biuret reaction (2.32 mg mL⁻¹) when bovine serum albumin (BSA) was used as the standard. The value obtained by quantitative amino acid analysis was 1.95 mg mL⁻¹. Wiechelman and coworker [37] showed that cysteine, cystine, tryptophan, tyrosine and the peptide bond are capable of reducing cupric ions to cuprous ions in the BCA reaction.

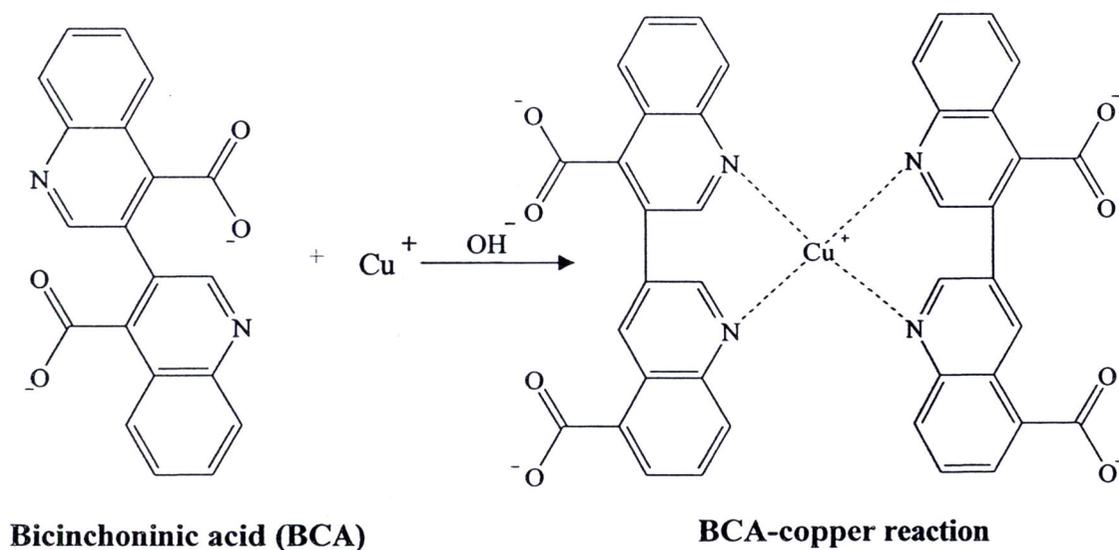


Figure 4 The reaction of BCA with cuprous ion [35]

The BCA Protein assay combines the protein-induced biuret reaction with the highly sensitive and selective colorimetric detection of the resulting cuprous cation

(Cu^+) by bicinchoninic acid (BCA). Thus, two steps are involved. First is the biuret reaction (Figure 2), whose faint blue color results from the reduction of cupric ion to cuprous ion. Second is the chelation of BCA with the cuprous ion (Figure 4), resulting in an intense purple color. The purple colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion. The BCA-copper complex is water-soluble and exhibits a strong linear absorbance at 562 nm with increasing protein concentrations. The purple color can be measured at any wavelength between 550 nm and 570 nm with minimal (less than 10%) loss of signal. The BCA reagent is approximately 100 times more sensitive (lower limit of detection) than the biuret reagent [35].

The Lowry method

The Lowry method was applied by suggesting the use of the Folin phenol reagent for the determination of protein concentration. The Lowry reaction is based on the amplification of the biuret reaction by subsequent reaction with the Folin phenol reagent (Folin–Ciocalteu reagent). Factors other than the biuret reaction play a role in the development of color in the Lowry method, resulting in considerable variation with respect to protein composition. This variation is a reflection of the contributions of specific amino acids (tyrosine, tryptophan) on color development in this reaction. The reaction of the Lowry method is involved as in Figure 5. A modification in which the detection wavelength is 650 nm instead of 750 nm has been described, which reduced protein variability. Various substances interfere with the Lowry reaction, including many nitrogen-containing buffers. A modification of the Lowry reaction that minimizes such interference has been reported [38].

As with the biuret reaction, the use of the Lowry reaction has decreased in recent years as more facile and sensitive protein assays have become available. However, there is still substantial use of this technique as evidenced by the following citations. The Lowry assay has been used by Williams and Halsey [39] to measure protein in latex gloves. Using this technique, protein levels ranged from less than 25 to 1150 $\mu\text{g g}^{-1}$ of glove. Other laboratories have also been used the Lowry method to measure protein in latex gloves [40, 41].

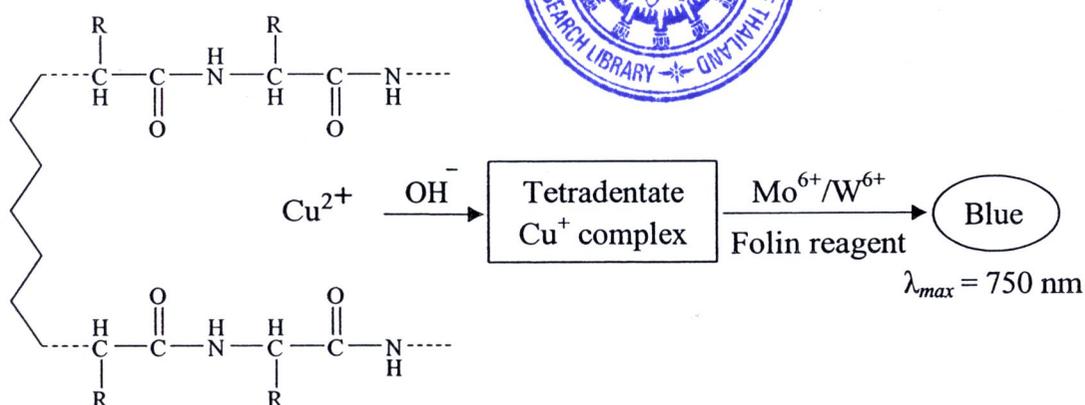


Figure 5 The schematic reaction for the Lowry Protein Assay [42]

Three laboratory assays (The Lowry test, immunochemical test and radioallergosorbent test) are currently available to determine the quantity of protein in a specimen. The Lowry test, a chemical method based on the binding of chromogenic dye to protein residues, is the least sensitive of the three methods but is currently the national standard (American Society for Testing and Materials, ASTM D5712). There are also two immunochemical tests: the Latex Enzyme Linked Immunosorbent Assay for Antigenic Protein (LEAP), which uses rabbit IgG, and the Radioallergosorbent Test (RAST), which is a human IgE inhibition assay. The ASTM is currently considering a new standard for glove protein levels based on the Enzyme-Linked Immunosorbent Assay (ELISA), which can detect protein levels $<50 \mu\text{g g}^{-1}$ minimum under the existing standard [19].

Digital image-based colorimetry (DIC)

In recent years, the advent of instrumental methods of analysis was performed on the basis of reagent-based colorimetric tests. There has been a rapid improvement on the technology of digital photography called digital image-based colorimetry (DIC) [43]. DIC is based on CCD and CMOS image sensor. A modification of CCD and CMOS camera as an analytical detector has increased the attention during the last decades. These were used for signal recording based on red, green, and blue (RGB) tricolor theory. The widespread applications of DIC were prediction of leaf chlorophyll content [44], water quality measurements [45], clinical measurement of blood glucose [46], identification of natural amino acids [47], and application in

analytical chemistry [48, 49, 50, 51]. Based on the RGB theory, CCD camera was used for the determination of total phosphorus in water samples using DIC [48]. The RGB values from the digital image were transformed into gray-scale format. It was found that the RGB values of different color gradients have a good linear relationship with the concentration of phosphorus standard solution. Suzuki and coworkers [49] used a digital still camera (DSC) as a colorimeter applied to the determination of Fe(II) and residual chlorine in river and tap water samples. A dedicated light-box containing white-color LEDs as light source was made of white acrylic to make constant exposure at each photograph and analyzed L^* (brightness), a^* (red-green component), and b^* (yellow-blue component) values from an image by a written program. Gaiao and coworkers [50] fabricated a light-box containing a CCD camera (webcam) used as a detector for acid-base titration. The RGB based value that is calculated for each digital image was monitored. It was found that the digital image based (DIB) titration is comparable with the spectrophotometric titration. In addition, a CMOS webcam camera was employed as a small and low cost detector demonstrated with a simple lab-on-chip (LOC) reactor for real time continuous monitoring of the color reaction of acid-base neutralization [51]. The fading of pink color of the indicator when the acidic solution diffused into the basic solution zone was recorded as the change of red, blue and green colors (%RBG). The results obtained show the successful performance of the simple LOC-CMOS system for acidity assay. Obviously, the use of digital imaging in analytical chemistry becomes possible.

Digital camera [52]

A digital camera is a camera that takes video or still photographs, or both, digitally by recording images via an electronic image sensor. It is the main device used in the field of digital photography. Most 21st century cameras are digital. Digital cameras can do things film cameras cannot: displaying images on a screen immediately after they are recorded, storing thousands of images on a single small memory device, and deleting images to free storage space. The majority, including most compact cameras, can record moving video with sound as well as still photographs. Some can crop and stitch pictures and performs other elementary image editing. Some have a GPS receiver built in, and can produce Geotagged photographs.

The optical system works the same as in film cameras, typically using a lens with a variable diaphragm to focus light onto an image pickup device. The diaphragm and shutter admit the correct amount of light to the imager, just as with film but the image pickup device is electronic rather than chemical. Most digital camera, apart from camera phones and a few specialized types, have a standard tripod screw. Digital cameras are incorporated into many devices ranging from PDA's and mobile phones to vehicles. The Hubble space telescope and other astronomical devices are essentially specialized digital cameras.

Image sensor

An image sensor is a device that converts an optical image into an electronic signal. It is used mostly in digital cameras and other imaging devices. Early analog sensors were video camera tubes, most currently used are digital charge-coupled device (CCD) or complementary metal-oxide-semiconductor (CMOS) active pixel sensors. Both CCD and CMOS technologies are based on arrays of light-sensitive pixels (or photosites), which gather photons of light and convert them to a visible image. How this process works influences the qualities and capabilities of the sensors.

CCD Pixel Arrays

CCD photosites as shown in Figure 6, arranged in an array, collect electrons when exposed to light [53]. They pass the electrons from one charge-collecting bucket to another charge-collecting bucket, traversing the entire array. This method allows for sequential readout and good output uniformity. Reading the values of each cell as they are transported across the chip uses significant amounts of power. It requires as much as 2.5 to 10 volt clocking signals and multiple supply and bias voltages. The high power consumption is necessary for the CCD to maintain its image quality and limit noise.



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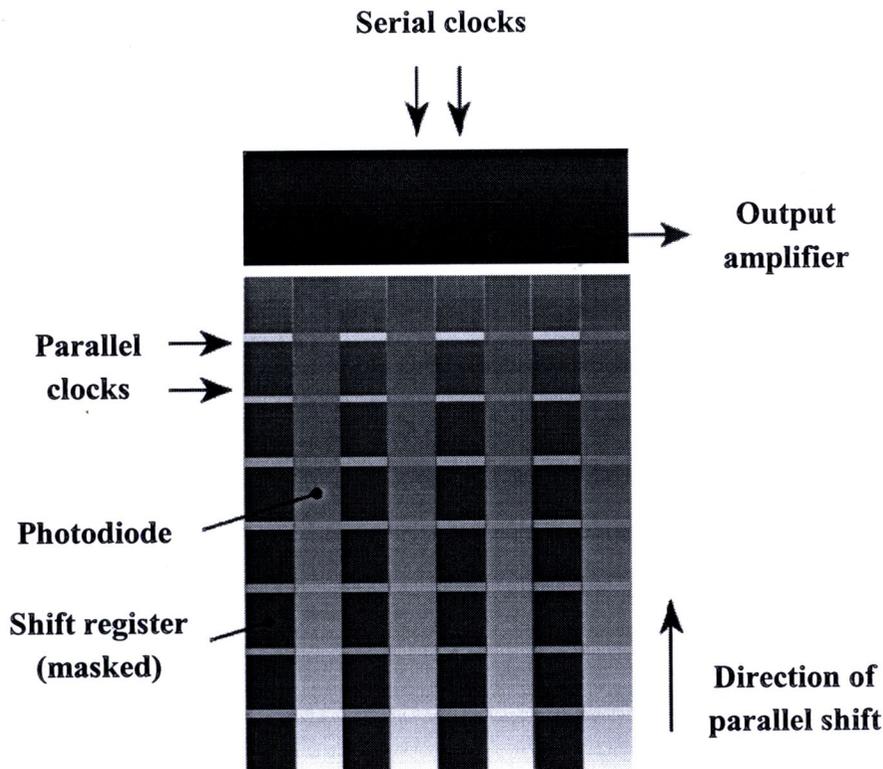


Figure 6 CCD pixel arrays [53]

Additionally, the process used to manufacture CCD chips keeps their fidelity and light sensitivity high, but it drives up production costs. CCD pixel arrays are optimized to lower noise and improve image quality. Because of their architecture, they require additional clock signals, converters, and controls off of the chip.

CMOS Pixel Arrays

CMOS sensors (Figure 7) often have three or four transistors in each photosite, which amplify and move the charge provided by incoming photons of light [53]. They enable the pixels to be read individually. Some of the light entering a given pixel lands on a transistor, not on the sensor's light-detecting photodiode, which means that the CMOS pixel might not be as sensitive as a CCD pixel. CMOS image sensors operate at a very low gain and also produce more noise than a CCD imager. However, the CMOS manufacturing process uses standard microprocessor technology, which lowers the production cost significantly and can make integration simpler. CMOS pixel arrays are fabricated with standard silicon processes, enabling peripheral electronics to be included on the chip.

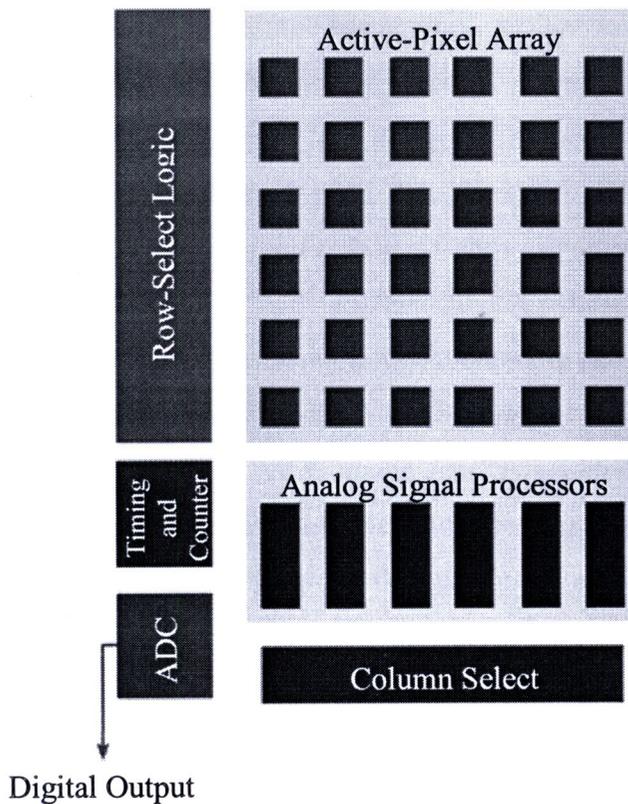


Figure 7 CMOS pixel arrays [53]

The difference between CCD and CMOS image sensors

CCD (charge coupled device) and CMOS (complementary metal oxide semiconductor) image sensors are two different technologies for capturing images digitally [54]. Each has unique strengths and weaknesses giving advantages in different applications. Neither is categorically superior to the other, although vendors selling only one technology have usually claimed otherwise. In the last five years much has changed with both technologies, and many projections regarding the demise or ascendance of either have been proved false. The current situation and outlook for both technologies is vibrant, but a new framework exists for considering the relative strengths and opportunities of CCD and CMOS imagers.

Both types of imagers convert light into electric charge and process it into electronic signals. In a CCD sensor, every pixel's charge is transferred through a very limited number of output nodes (often just one) to be converted to voltage, buffered, and sent off-chip as an analog signal. All of the pixel can be devoted to light capture, and the output's uniformity (a key factor in image quality) is high. In a CMOS sensor,

each pixel has its own charge-to-voltage conversion, and the sensor often also includes amplifiers, noise-correction, and digitization circuits, so that the chip outputs digital bits. These other functions increase the design complexity and reduce the area available for light capture. With each pixel doing its own conversion, uniformity is lower. But the chip can be built to require less off-chip circuitry for basic operation [7, 55].

CCDs and CMOS imagers were both invented in the late 1960s and 1970s. CCD became dominant, primarily because they gave far superior images with the fabrication technology available. CMOS image sensors required more uniformity and smaller features than silicon wafer foundries could deliver at the time. Not until the 1990s did lithography develop to the point that designers could begin making a case for CMOS imagers again. Renewed interest in CMOS was based on expectations of lowered power consumption, camera-on-a-chip integration, and lowered fabrication costs from the reuse of mainstream logic and memory device fabrication. While all of these benefits are possible in theory, achieving them in practice while simultaneously delivering high image quality has taken far more time, money, and process adaptation than original projections suggested (see Table 2).

The differences between CCD and CMOS image sensors are shown in Table 3. Both CCDs and CMOS imagers can offer excellent imaging performance when designed properly. CCDs have traditionally provided the performance benchmarks in the photographic, scientific, and industrial applications that demand the highest image quality (as measured in quantum efficiency and noise) at the expense of system size. CMOS imagers offer more integration (more functions on the chip), lower power dissipation (at the chip level), and the possibility of smaller system size, but they have often required tradeoffs between image quality and device cost. Today there is no clear line dividing the types of applications each can serve. CMOS designers have devoted intense effort to achieving high image quality, while CCD designers have lowered their power requirements and pixel sizes. As a result, you can find CCDs in low-cost low-power cellphone cameras and CMOS sensors in high-performance professional and industrial cameras, directly contradicting the early stereotypes. It is worth noting that the producers succeeding with "crossovers" have almost always been established players with years of deep experience in both technologies.

Costs are similar at the chip level. Early CMOS proponents claimed CMOS imagers would be much cheaper because they could be produced on the same high-volume wafer processing lines as mainstream logic or memory chips. This has not been the case. The accommodations required for good imaging performance have required CMOS designers to iteratively develop specialized, optimized, lower-volume mixed-signal fabrication processes, very much like those used for CCDs. Proving out these processes at successively smaller lithography nodes (0.35 μm , 0.25 μm , 0.18 μm) has been slow and expensive; those with a captive foundry have an advantage because they can better maintain the attention of the process engineers.

Table 2 CMOS development's winding path [54]

Initial prediction for CMOS	Twist	Outcome
Equivalence to CCD in imaging performance	Required much greater process adaptation and deeper submicron lithography than initially thought	High performance available in CMOS, but with higher development cost than CCD
On-chip circuit integration	Longer development cycles, increased cost, tradeoffs with noise, flexibility during operation	Greater integration in CMOS, but companion chips still required for both CMOS and CCD
Reduced power consumption	Steady improvement in CCDs	Advantage for CMOS, but margin diminished
Reduced imaging subsystem size	Optics, companion chips and packaging are often the dominant factors in imaging subsystem size	CCDs and CMOS comparable

Table 2 (Cont.)

Initial prediction for CMOS	Twist	Outcome
Economies of scale from using mainstream logic and memory foundries	Extensive process development and optimization required	CMOS imagers use legacy production lines with highly adapted processes akin to CCD fabrication

Table 3 Feature and performance comparison [54]

Feature	CCD	CMOS
Signal out of pixel	Electron packet	Voltage
Signal out of chip	Voltage (analog)	Bits (digital)
Signal out of camera	Bits (digital)	Bits (digital)
Fill factor	High	Moderate
Amplifier mismatch	N/A*	Moderate
System Noise	Low	Moderate
System Complexity	High	Low
Sensor Complexity	Low	High
Camera components	Sensor + multiple support chips + lens	Sensor + lens possible, but additional support chips common
Relative R&D cost	Lower	Higher
Relative system cost	Depends on Application	Depends on Application
Performance	CCD	CMOS
Responsivity	Moderate	Slightly better
Dynamic Range	High	Moderate

Table 3 (Cont.)

Feature	CCD	CMOS
Uniformity	High	Low to Moderate
Uniform Shuttering	Fast, common	Poor
Uniformity	High	Low to Moderate
Speed	Moderate to High	Higher
Windowing	Limited	Extensive
Antiblooming	High to none	High
Biasing and Clocking	Multiple, higher voltage	Single, low-voltage

*N/A = Not available

CMOS cameras may require fewer components and less power, but they still generally require companion chips to optimize image quality, increasing cost and reducing the advantage they gain from lower power consumption. CCD devices are less complex than CMOS, so they cost less to design. CCD fabrication processes also tend to be more mature and optimized; in general, it will cost less (in both design and fabrication) to yield a CCD than a CMOS imager for a specific high-performance application. However, wafer size can be a dominating influence on device cost; the larger the wafer, the more devices it can yield, and the lower the cost per device. 200 mm is fairly common for third-party CMOS foundries while third-party CCD foundries tend to offer 150 mm. Captive foundries use 150 mm, 200 mm, and 300 mm production for both CCD and CMOS.

The larger issue around pricing is sustainability. Since many CMOS startups pursued high-volume, commodity applications from a small base of business, they priced below costs to win business. For some, the risk paid off and their volumes provided enough margin for viability. But others had to raise their prices, while still others went out of business entirely. High-risk startups can be interesting to venture capitalists, but imager customers require long-term stability and support.

While cost advantages have been difficult to realize and on-chip integration has been slow to arrive, speed is one area where CMOS imagers can demonstrate considerable strength because of the relative ease of parallel output structures. This gives them great potential in industrial applications. CCDs and CMOS will remain complementary. The choice continues to depend on the application and the vendor more than the technology.

Types of digital cameras

Point and shoot cameras

A point-and-shoot camera (Figure 8), also called a compact camera, is a still camera designed primarily for simple operation. Most use focus free lenses or autofocus for focusing, automatic systems for setting the exposure options, and have flash units built in [56].



Figure 8 Point and shoot cameras [57]

Camera phone

A camera phone (Figure 9) is a mobile phone which is able to capture still photographs (and usually video). Most camera phones are simpler than separate digital cameras. Their usual fixed focus lenses and smaller sensors limit their performance in poor lighting. Having no physical shutter, most have a long shutter lag. Most have no flash or optical zoom [58] or tripod screw. Many lack a USB connection, removable memory card, or other way of transferring their pictures more quickly than by the phone's inherent communication feature [59].



Figure 9 Camera phone [60]

One-time-use cameras

The disposable or single-use camera (Figure 10) is a simple box camera sold with a roll of film installed, meant to be used once. Most use fixed-focus lenses. Some are equipped with an integrated flash unit, and there are even waterproof versions for underwater photography [61]. Digital one-time-use cameras (and also digital one-time-use camcorders) are available in some markets; for example the US saw the introduction of a digital camera in 2004 [62]. Digital disposables have not had the success of their film based counterparts, possibly from the expense of the process (especially compared to normal digital camera use) and the poor quality of the images compared to either a typical digital camera, or a disposable film camera. Usually, the display shows the number of shots remaining, and once this is completed, the camera is returned to the store. The digital files are then extracted from the camera, and in return for keeping the camera, they are printed out or stored to CD (or DVD in the case of the Video Camera [63]) for the customer.



Figure 10 Disposable camera [62]

High-end fixed lens cameras

A fixed lens camera (Figure 11) is an advanced digital camera that looks like a digital single lens reflex (DSLR), but that cannot use interchangeable lenses. Fixed lens cameras typically offer very large zoom lenses, and they usually cost more than point and shoot, beginner models. Some fixed lens cameras can slightly add to their zoom and wide angle capabilities through the use of conversion lenses. Even though fixed lens cameras don't fit the exact definition of a DSLR model, some people refer to fixed lens cameras as "beginner" DSLR cameras or DSLR-style cameras and include them in the DSLR class. Such cameras can serve as a bridge for an intermediate photographer looking to migrate from a beginner camera to a DSLR [64].



Figure 11 High-end fixed lens cameras [65]

Digital single-lens reflex camera (digital SLR or DSLR)

Digital single-lens reflex cameras (Figure 12) are digital cameras that use a mechanical mirror system and pentaprism to direct light from the lens to an optical viewfinder on the back of the camera. Due to their reflex design system, DSLRs are often preferred by professional still photographers because they allow an accurate preview of framing close to the moment of exposure. Many professionals also prefer DSLRs for their larger sensors compared to most compact digital cameras. DSLRs have sensors which are generally closer in size to the traditional film formats that many current professionals started out using. These large sensors allow for similar depths of field and picture angle to film formats, as well as their comparatively high signal to noise ratio. DSLRs also allow the user to choose from a variety of

interchangeable lenses. Most DSLRs also have a function that allows accurate preview of depth of field [66].



Figure 12 Digital single-lens reflex camera [67]

Video camera

A video camera (Figure 13) is a camera used for electronic motion picture acquisition, initially developed by the television industry but now common in other applications as well [68].



Figure 13 Video camera [69]

A webcam (Figure 14) is a video camera that feeds its images in real time to a computer or computer network, often via USB, Ethernet, or Wi-Fi. Their most popular use is the establishment of video links, permitting computers to act as videophones or videoconference stations. This common use as a video camera for the World Wide Web gave the webcam its name. Other popular uses include security surveillance and computer vision and there are also uses on sites like video broadcasting services and for recording social videos. Webcams are known for their low manufacturing cost and flexibility, making them the lowest cost form of video telephony. They have also

become a source of security and privacy issues, as some built-in webcams can be remotely activated via spy ware [70].



Figure 14 Webcam [71]

Color theory [72]

Throughout history, attempts have been made to ascertain how colors are created, and what happens when various colors are mixed. The ancient greeks developed theories as far back as 350 BC to describe how to create various colors, which were largely based on the fact that colors are only visible in light that is brighter than black or darker than the sun. From this, they surmised that colors must be dependent on the amount of darkness or lightness present.

In 1704, Sir Isaac Newton challenged these beliefs when he published *Opticks* in which he demonstrated his “color circle” and proposed that color is actually a fundamental property of light itself, rather than a physical property of objects emitting color. Newton’s color circle was formed by taking the colors of the visible spectrum and wrapping them into a circle so that the red end joined the blue end. Using this device he was able to demonstrate a number of relationships between the colors including the fact that mixing a small number of colors could result in white light.

Since Newton’s time, color theorists have revised and developed color wheels into the endless variety and complexity that can be found today. Generally color wheels take two forms, those that describe the results obtained when mixing colors in the form of dyes or paints, and those, like Newton’s, which describe the effects of mixing light.

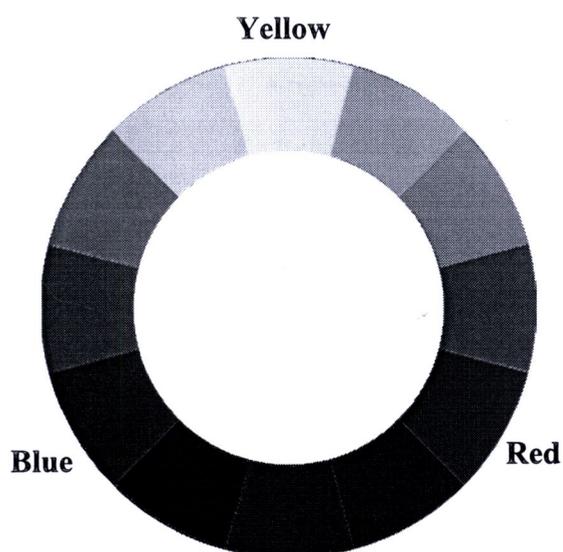


Figure 15 The artist's color wheel [73]

The artist's color wheel (Figure 15) is based on three fundamental or primary colors; Red, Yellow and Blue which are considered as such because they cannot be made by mixing other dyes, paints or pigments. This is the most common kind of color wheel you are likely to encounter. For these purposes, in the modern age of digital color, this work will concentrate on the "RGB" color wheel which uses red, green and blue as its basic colors from which all others are created (Figure 16).

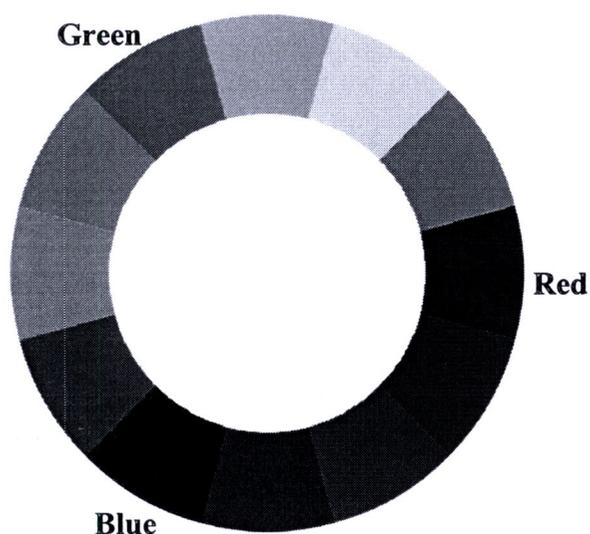


Figure 16 The RGB color wheel [74]

The RGB color wheel shown above contains 12 colors, arranged in a circle where each color is created by mixing the two adjacent to it. The colors red green and blue are used by electronic devices such as computer screens, televisions, and of course cameras to create all the myriad colors required in full color imagery. These three colors are fundamental building blocks used in the creation of color. By analyzing the positions of all the colors on the color wheel, it is possible to learn a great deal about colors and their relationships to each other.

The primary colors

Red, green and blue (Figure 17(a)) are the building blocks that are used to create all the other colors on the RGB color wheel. These colors are fundamental and are known as primary colors.

The secondary colors

Positioned half way between each pair of primary colors are the secondary colors, yellow, cyan and magenta (Figure 17(b)). These colors are created by mixing equal amounts of the two primaries closest to them. For example, cyan is created by mixing an equal amount of blue and green. It is also worth noting that each secondary color does not contain any of the color opposite it on the color wheel. Therefore, yellow contains no blue, only red and green, and for this reason it is sometimes known as “minus blue”.

The tertiary colors

To complete the color wheel, colors in the positions between the Primary and Secondary colors are inserted. These colors are created by mixing those colors adjacent to them so for example between red and yellow is orange which is created by mixing red and yellow equally. Although, a name for the mixture of red and yellow is orange, some of the other tertiary colors do not have their own names. Consequently, it is more normal to name the tertiary colors by the colors that are used to create them. Hence, these are Red-Yellow, Green-Yellow, Green-Cyan, Blue-Cyan, Blue-Magenta and Red-Magenta (where all the names take the form primary-secondary) (Figure 17(c)).

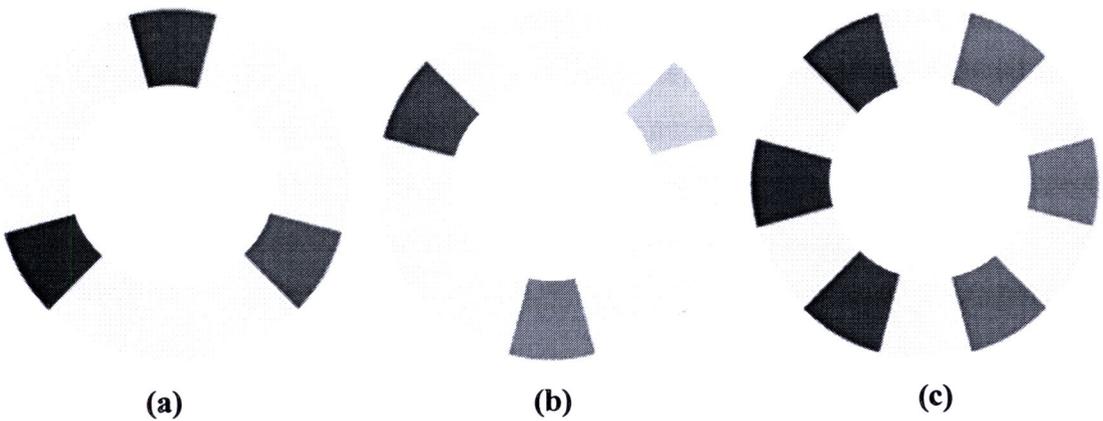


Figure 17 Primary (a), secondary (b) and tertiary (c) colors [72]

Hue, Saturation and Value (HSV)

At this point it is worth noting that all the colors are pure colors in that they contain no white or black. The term used to describe such colors is “Hue”, which can be considered as the dominant wavelength in any light source. Red, blue, green and yellow are examples of Hues, whereas pink and scarlet both have the Hue Red.

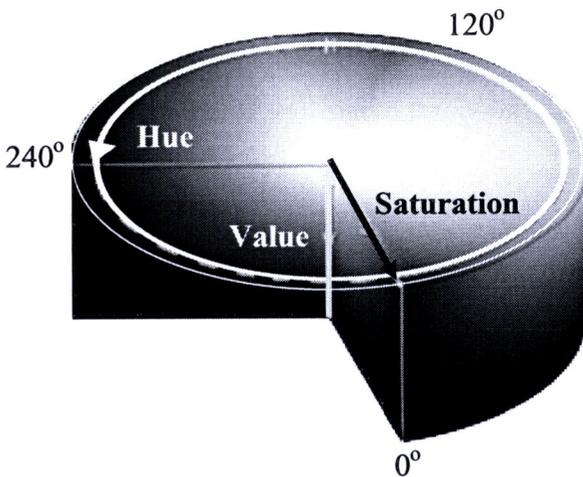


Figure 18 The HSV color model [75]

Imagine tipping the color wheel on its side and then extending it to three dimensions (Figure 18), so that it forms a cylindrical shape. In the centre of the wheel on the top surface of the cylinder is white and at the center of the bottom surface is black. Throughout the depth of the cylinder at the centre of the circles are all the infinite tones ranging from white to black. When colors are mixed with white (or made

lighter) they are no longer of pure Hue, and are known as “Tints”. When mixed with black (or made darker) they are known as “Shades”. The amount of white, black or grey that is mixed with any Hue defines its “Saturation” and colors near the central axis of the cylinder are “de-saturated”. The lightness or darkness of any color is dependent on the tonality of white black or grey mixed with it. This tonality is known as the “Value” by using the three values of Hue, Saturation and Value. It is possible to precisely define any possible color.

Complementary Colors

From the relationships between colors, how they interact with each other and various properties of specific sections of the color wheel. Any two colors opposite each other on the color wheel are known as “Complementary Colors”. In black and white photography, the key compositional components are tone, texture and form. In color photography we add to this list color, and complementary colors provide the greatest “Color Contrast”. By limiting the colors used in any composition to two complementary colors, we maximize the color contrast, which gives greater impact to our images. It doesn’t matter which two complementary colors we choose, blue and yellow, green and magenta or red and cyan (Figure 19), any combination of two colors on opposite sides of the color wheel will make the colors used appear more vibrant, particularly if they are of pure Hue.

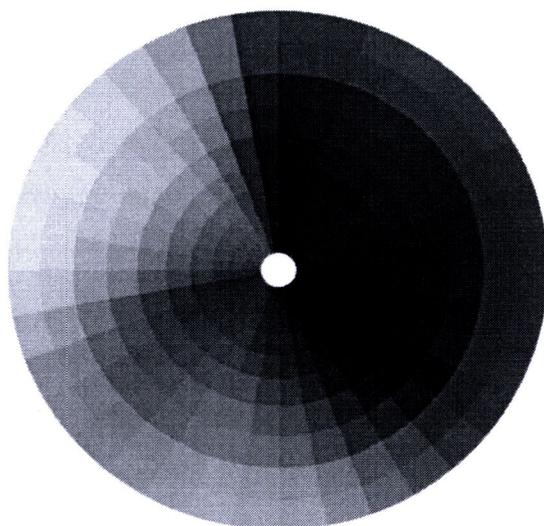


Figure 19 Complementary colors [76]

The Color Model [77, 78]

Color models, like all mathematical representations of physical phenomena, can be expressed in many different ways, each with its advantages and drawbacks. Some representation are formulated to help humans select colors, the Munsell system and others are formulated to ease data processing in machines, with the various RGB spaces all falling in this last category. The goal is to minimize formulation complexity and the number of variables while maximizing “substance” and breath of coverage. One thing they have in common is the number of variables, or dimensions. Historically, whatever the meaning assigned to the variables, three of them were enough to describe all colors: RGB, Hue-Saturation-Brightness (HSB) and other HS based models, $L^*a^*b^*$, xyY , etc. From this observation alone, one would be tempted to conclude that color is perceived with a three signal output mechanism to the brain since nature often uses a minimalist approach to do things. In many cases, more variables are added to complete a theory’s coverage or to supplement a physical limitation of the reproduction process. For example, black content (“K”) is added to cyan, magenta and yellow (CMY) inks to obtain better dark tones in traditional printing. Printing processes with more than four colors such as; Pantone Hexachrome with six colors and HiFi color with up to eight colors have been developed to extend the reproducible color range. Some desktop printers are now offered with two additional color cartridges, consisting of light cyan and light magenta, which are designed to improve color gradients uniformity in the highlights, where print density would be low, and the dots visible, for normally concentrated cyan and magenta inks. These added variables are not additional dimensions per se since they are not totally independent of the primary coordinates. Some of the colors generated by mixing the additional inks with the primaries can also be generated by mixing only the original primaries.

RGB and its subset CMYK form the most basic and well-known color model. This model bears closest resemblance to how to perceive color. It also corresponds to the principles of additive and subtractive colors.

1. Additive Colors

The modern understanding of the properties of light and color owes a great deal to the pioneering work carried out by Sir Isaac Newton at the latter end of

the seventeenth century. Newton showed that a prism could break white light down into its component colors, and that a further arrangement of prisms could refract the light back in to its original form. In his publication OPTIKS, he famously stated “For the rays to speak properly are not colored”, his way of saying that color as a property is a psychological phenomenon and not a physical one. An additive color system relates to light that is emitted. It does not refer to an object that has the property red only to a light source that is emitting red light. An additive system requires three light sources, each emitting one of the primary colors red, green and blue. If two of these light sources were mixed in equal amounts, the resulting color is one of the secondary colors cyan, yellow and magenta. When mixing equal amounts of all three light sources the result is white light.

The additive color system using colored light sources, confirms the facts discovered in the section on the color wheel. Yellow is made from red and green and contains no blue. It should also be clear that a mixture of red and green light in equal amounts (light of two very different wavelengths) is physically different from pure yellow light (with a single wavelength of roughly 580 nm). The important thing to realize is that both stimulate our eyes and brains to give the same result that is a psychological impression that we call “yellow”.

The brilliant Scottish physicist James Clerk Maxwell is considered to be the founder of additive color synthesis. In 1861, Maxwell presented to the Royal Society in London a demonstration based on ideas he had introduced at the Royal Society of Edinburgh some 6 years earlier. He had arranged for the photographer Thomas Sutton to photograph a tartan ribbon 3 times (using the available black and white film), each time with a different filter over the lens; red, green and blue. The three images were then projected onto the same screen and each projector lens was covered with the same color filter used to take the original image. When the three projections were brought into register, a full color image was seen to appear. As a footnote to Maxwell’s demonstration, we now know that he was extremely lucky that it worked at all. The black and white film used by Sutton was only sensitive to light from the blue end of the spectrum and shouldn’t have recorded anything through the red and green filters. An attempt to repeat the experiment nearly 100 years later showed that Maxwell’s green filter had in fact allowed some blue light through, and

that the ribbon's red colors were reflecting ultraviolet which was recorded with the red filter in place.

CMYK color

Cyan, magenta, and yellow correspond roughly to the primary colors in art production: red, blue, and yellow. In the illustration below, you can see the CMY counterpart to the RGB model shown in Figure 20:

Just as the primary colors of CMY are the secondary colors of RGB, the primary colors of RGB are the secondary colors of CMY. But as the illustrations show, the colors created by the subtractive model of CMY are not actually like the colors created in the additive model of RGB. Particularly, CMY cannot reproduce the brightness of RGB colors.

In theory, the combination of cyan, magenta, and yellow at 100%, create black (all light being absorbed). In practice, however, CMY usually cannot be used alone. Due to imperfections in the inks and other limitations of the process, full and equal absorption of the light isn't possible; thus a true black or true grays cannot be created by mixing the inks in equal proportions. The actual result of doing so results in a muddy brown color. In order to boost grays and shadows, and provide a genuine black, printers resort to adding black ink, indicated as K. Thus the practical application of the CMY color model is the four color CMYK process.

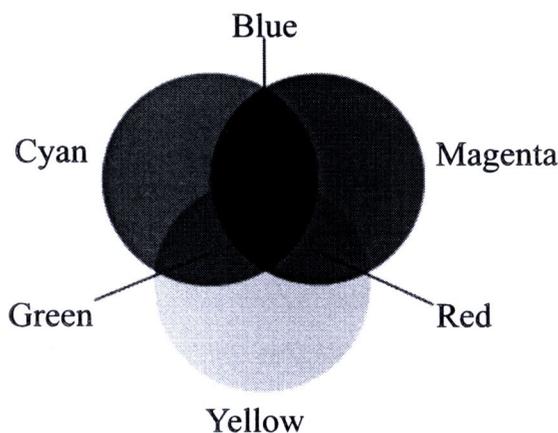


Figure 20 CMYK Color [77]

2. Subtractive Color

Subtractive color theory explains how to mix inks, dyes, paints and natural colorants to create colors which absorb some wavelengths of light and reflect others. An object that absorbs one of the three primary colors from the additive world (perhaps red) will reflect the other two (green and blue) and appear to be the color of the reflected light (in this case Cyan). It follows from this that an object that absorbs Cyan is effectively absorbing Green and Blue and will therefore reflect only Red. The color of any object viewed under white light is determined by the wavelengths of light its surface reflects and absorbs.

When viewed under a white light, a nice ripe banana appears Yellow. This isn't because it emits Yellow light (the additive world) but because it absorbs Blue light and reflects only yellow. Without the white light to illuminate the banana, it would be in the dark and would appear to have no color at all. The primary colors of the subtractive world are Cyan, Magenta and Yellow, so it should come as no surprise that the secondary colors are now Red, Green and Blue. Cyan ink for example absorbs Green and Blue light and can therefore be used to control how much red is reflected. By altering the amount of each ink that is placed on the paper, it is theoretically possible to produce almost any color. In practice, however, impurities in the manufacture of inks mean that it is impossible to create true blacks.

When mixing Cyan, Magenta and Yellow, all that can realistically be achieved is a muddy brown. For this reason, a fourth color ink, Black, is added to the process. The CMY inks are used to create the Hue and the Black (K) ink is used to control the Value. The subtractive printing process is often referred to as CMYK after the four color inks used in printing.

RGB color

Red, green, and blue are the primary stimuli for human color perception and are the primary additive colors. The relationship between the colors can be seen in Figure 21.

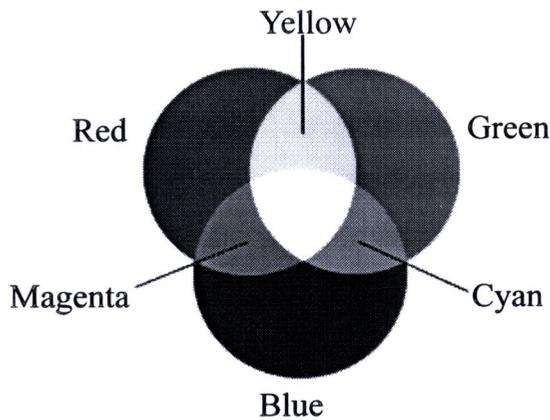


Figure 21 RGB color [78]

The secondary colors of RGB, cyan, magenta, and yellow, are formed by the mixture of two of the primaries and the exclusion of the third. Red and green combine to make yellow, green and blue make cyan, blue and red make magenta. The combination of red, green, and blue in full intensity makes white. White light is created when all colors of the electromagnetic spectrum converge in full intensity. The importance of RGB as a color model is that it relates very closely to the way we perceive color with the receptors in our retinas. RGB is the basic color model used in television or any other medium that projects the color. It is the basic color model on computers and is used for Web graphics, but it cannot be used for print production. RGB colors are defined using a hexadecimal notation (HEX) for the combination of Red, Green, and Blue color values (RGB). The lowest value that can be given to one of the light sources is 0 (in HEX: 00). The highest value is 255 (in HEX: FF). The combination of Red, Green, and Blue values from 0 to 255, gives more than 16 million different colors ($256 \times 256 \times 256$).

Neural networks

The term neural network was traditionally used to refer to a network or circuit of biological neurons [79]. The modern usage of the term often refers to artificial neural networks, which are composed of artificial neurons or nodes. Thus the term has two distinct usages [80]:

1. Biological neural networks [81]

The biological network consists of nerve cells (neurons) as illustrated in Figure 22, which are interconnected as in Figure 23. The cell body of the neuron, which includes the neuron’s nucleus is where most of the neural “computation” takes place. Neural activity passes from one neuron to another in terms of electrical triggers which travel from one cell to other down the neuron’s axon, by means of an electrochemical process of voltage-gated ion exchange along the axon and diffusion of neurotransmitter molecules through the membrane over the synaptic gap (Figure 24). The axon can be viewed as a connection wire. However, the mechanism of signal flow is not via electrical conduction but via exchange that is transported by diffusion of ions. This transportation process moves along the neuron’s cell, down the axon and then through synaptic junctions at the end of axon via a very narrow synaptic space to the dendrites and/or soma of next neuron at an average rate of 3 m sec^{-1} .

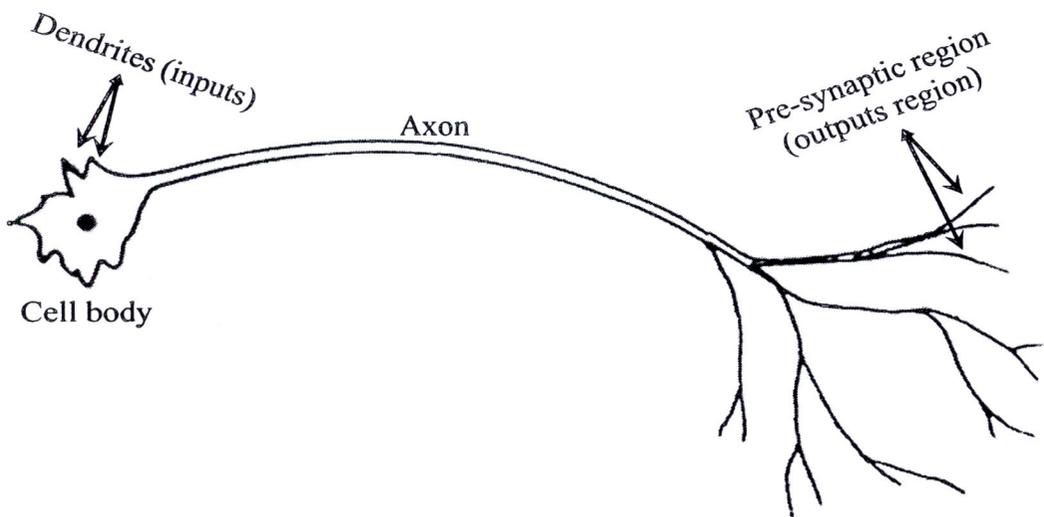


Figure 22 A biological neural cell (neuron) [81]

Figures 22 and 23 indicate that since a given neuron may have several (hundreds of) synapses, a neuron can connect (pass its message/signal) to many (hundred of) other neurons. Similarly, since there are many dendrites per each neuron, a single neuron can receive messages (neural signals) from many other neurons. In this manner, the biological neural network interconnects [82].

It is important to note that not all interconnections are equally weighted. Some have a higher priority (a higher weight) than others. Also some are excitatory and some are inhibitory (serving to block transmission of a message). These differences are affected by differences in chemistry and by the existence of chemical transmitter and modulating substances inside and near the neurons, the axons and in the synaptic junction. This nature of interconnection between neurons and weighting of messages is also fundamental to artificial neural networks (ANNs).

A simple analog of the neural element of Figure 22 is as in Figure 25. In that analog, which is common building block (neuron) of every artificial neural network, the differences in weighting of messages at the various interconnections (synapses) as mentioned above were observed. Analogs of cell body, dendrite, axon and synaptic junction of biological neural of Figure 22 are indicated in the appropriate parts of Figure 25. The biological network as represented in Figure 23 thus becomes the network of Figure 26.

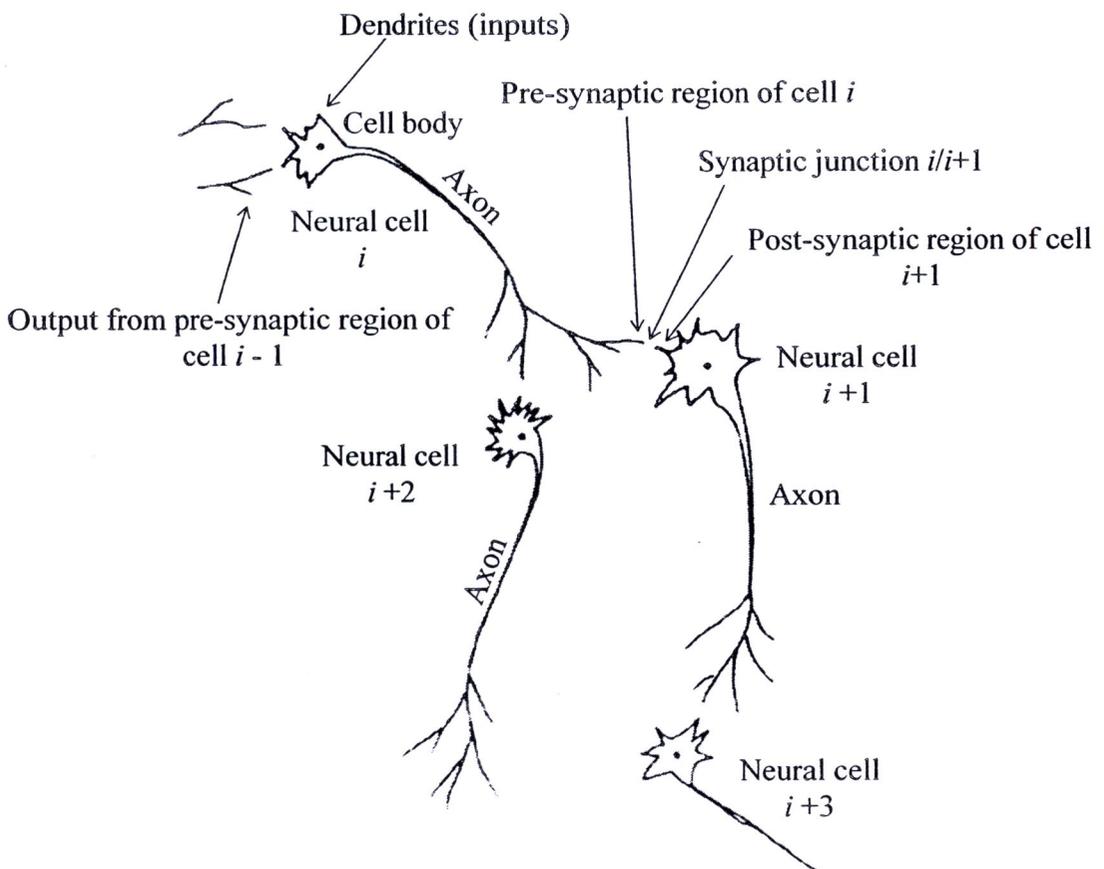


Figure 23 Interconnection of biological neural nets [81]

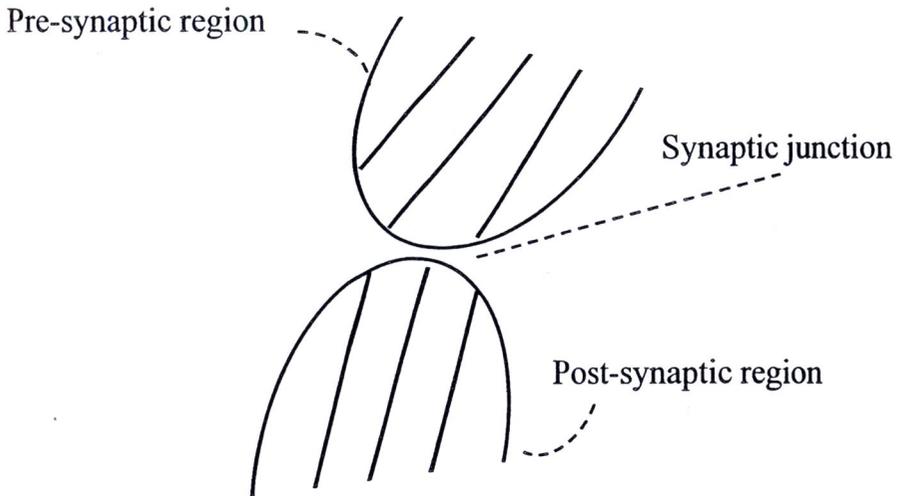


Figure 24 Synaptic junction (details of Figure 23) [81]

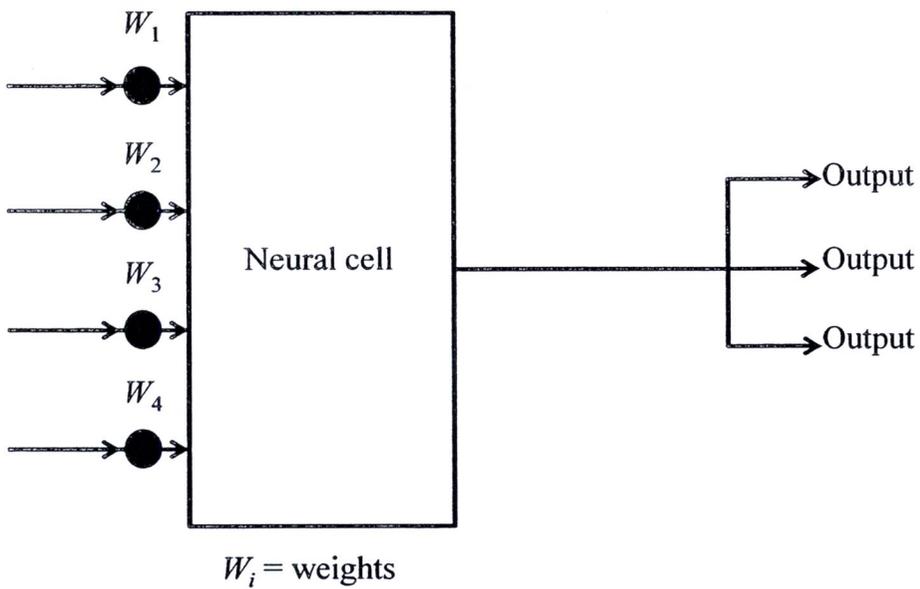


Figure 25 Schematic analog of a biological neural cell [81]

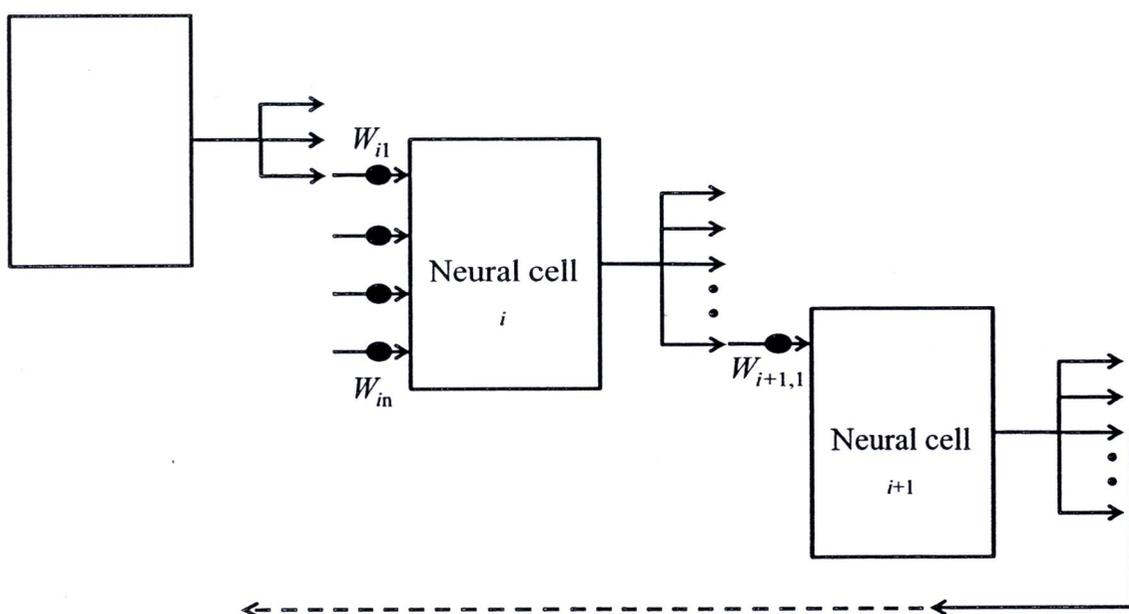


Figure 26 Schematic analog of a biological neural network [81]

2. Artificial neural networks [81]

Artificial neural networks (ANNs) are computational networks which attempt to simulate, in a gross manner, the networks of nerve cell of the biological (human or animal) central nervous system. This simulation is a gross cell-by-cell (neuron-by-neuron, element-by-element) simulation. It borrows from the neurophysiological knowledge of biological neurons and of network of such biological neurons. It thus differs from conventional (digital or analog) computing machines that serve to replace, enhance or speed-up human brain computation without regard to organization of the computing elements and of their networking.

The complexity of real neurons is highly abstracted when modeling artificial neurons. These basically consist of inputs (like synapses), which are multiplied by weights (strength of the respective signals), and then computed by a mathematical function which determines the activation of the neuron. Another function (which may be the identity) computes the output of the artificial neuron (sometimes in dependence of a certain threshold). ANNs combine artificial neurons in order to process information as shown in Figure 27.

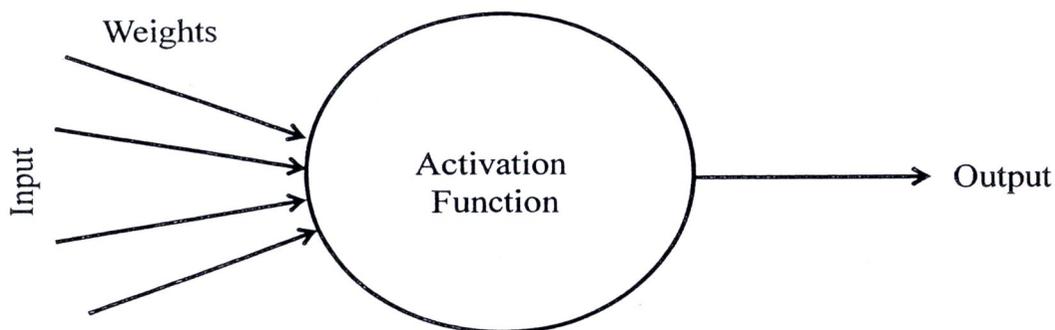


Figure 27 An artificial neuron [81]

The higher a weight of an artificial neuron is, the stronger the input which is multiplied by it will be. Weights can also be negative, so we can say that the signal is inhibited by the negative weight. Depending on the weights, the computation of the neuron will be different. By adjusting the weights of an artificial neuron, the output we want for specific inputs was obtained. But for an ANN of hundreds or thousands of neurons, it would be quite complicated to find by hand all the necessary weights. But, these could be algorithms which can adjust the weights of the ANN in order to obtain the desired output from the network. This process of adjusting the weights is called learning or training.

The number of types of ANNs and their uses is very high. Since the first neural model by McCulloch and Pitts [82] there have been developed hundreds of different models considered as ANNs. The differences in them might be the functions, the accepted values, the topology and the learning algorithms [83]. In addition, the properties of hybrid models is used in many applications. Therefore, in this review, only an ANN which learns using the backpropagation algorithm is presented because it is one of the most common models applied in chemistry [8, 87-90].

Since the function of ANNs is to process information, they are used mainly in fields related with it. There are a wide variety of ANNs that are used to model real neural networks, and study behavior and control in animals and machines, but also there are ANNs which are used for engineering purposes, such as pattern recognition, forecasting, and data compression.

Back-propagation algorithm

Back-propagation algorithm [84] is a widely used learning algorithm in Artificial Neural Networks. The Feed-Forward Neural Network architecture (Figure

28) is capable of approximating most problems with high accuracy and generalization ability. This algorithm is based on the error-correction learning rule. Error propagation consists of two passes through the different layers of the network, a forward pass and a backward pass. In the forward pass, the input vector is applied to the sensory nodes of the network and its effect propagates through the network layer by layer. Finally, a set of outputs is produced as the actual response of the network. During the forward pass, the synaptic weight of the networks are all fixed. During the back pass, the synaptic weights are all adjusted in accordance with an error-correction rule. The actual response of the network is subtracted from the desired response to produce an error signal. This error signal is then propagated backward through the network against the direction of synaptic conditions. The synaptic weights are adjusted to make the actual response of the network move closer to the desired response [85].

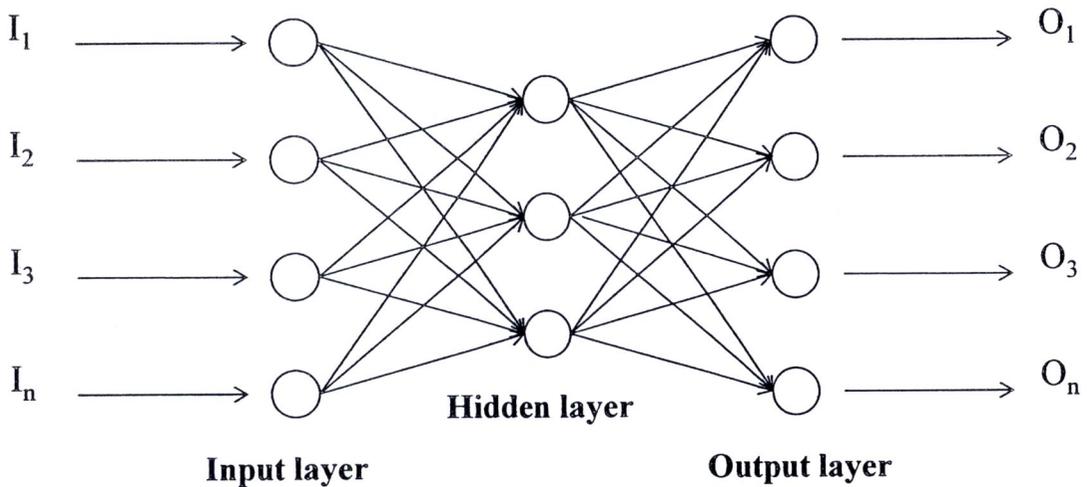


Figure 28 Feed-forward neural network architecture [86]

After choosing the weights of the network randomly, the back-propagation algorithm is used to compute the necessary corrections. The algorithm can be decomposed in the following four steps:

1. Feed-forward computation
2. Back-propagation to the output layer
3. Back-propagation to the hidden layer
4. Weight updates

The algorithm is stopped when the value of the error function has become sufficiently small.

Application of ANNs in analytical chemistry

In recent years, the advent of processing methods of analysis was performed on the basis of the usage of an ANNs. Maleki and coworkers [8] used CCD camera for signal recording in simultaneous determination of Al(III) and Fe(III) in synthetic alloys using the chrome azurol S (CAS) as chromogenic reagent. The RGB values associated to digital images from Al(III)-CAS and Fe(III)-CAS complexes were used in the construction of a back propagation neural network (BPNN) model. This method was also applied satisfactorily to the simultaneous determination of considered metal ions in two synthetic alloys. Besides, the digital colorimetric study was applied to colorimetric reaction for the analysis of Ti in representative plastics [87]. The reaction of Ti(IV) with hydrogen peroxide in acid media produced a yellow–orange complex solution. The apparent colors were determined by the combination of RGB data. The total intensity defined as R+G+B, represents the amplitude of the color space in each measurement. Calibration by ANNs represents an option that improves the answer to low concentration. The method is reduces the analysis time. Chuang and coworkers [88] applied a BPNN for human serum α -fetoprotein (AFP) measurement by combination with immuno-chromatographic strip technique. The color on the strip was recorded by using optical scanner. The 24-bit color images on the strip were converted to binary images (black and white) then processing by an ANNs written software. The newly constructed quantitative immunochromatographic strip assay is a simple, rapid, and reliable method for serum AFP measurement. Afkhami and coworkers [89] applied an ANNs in simultaneous determination of Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} with 3,6-bis((aminoethyl)thio)pyridazine by inputting the absorbance values in the wavelength range from 300-500 nm. Under the working conditions, the proposed methods were successfully applied to simultaneous determination of Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} in different vegetable, foodstuff and pharmaceutical product samples. A method for simultaneous analysis of V^{4+} and Co^{2+} has been developed by Safavi and coworkers [90]. This method is based on the difference of the chemical reaction rate of V^{4+} and Co^{2+} with Fe^{3+} in the presence of chromogenic reagent, 1,10-phenanthroline. The reduced product of the reaction, Fe^{2+} , can form a colored complex

with 1,10-phenanthroline and make a visible spectrophotometric signal for indirect monitoring of the V^{4+} and Co^{2+} concentrations. Feed forward neural networks have been trained to quantify considered metal ions in mixtures under optimum conditions. The proposed method was also applied satisfactorily to the determination of considered metal ions in several synthetic and water samples. Obviously, the use of digital imaging in analytical chemistry becomes possible. To the best of our knowledge, there was no report on the use of ANNs in combination with DIC as a detector for protein assay in NR latex and medical latex gloves. Therefore, in this work DIC-ANNs will be used and tested for the determination of protein in NR latex and medical latex gloves.

