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Research Article

Emerging Technologies for Fluorescence-Based Optical Test Strip Readers

Seda Aksoy[1], Ayse Dulda[2], Gokhan Ertas[*3]

[1] Yeditepe University, Graduate School of Natural and Applied Sciences, Department of Biomedical Engineering, İstanbul, Turkey, (ORCID: 0000-0002-3512-1932), seda.aksoy1@std.yeditepe.edu.tr
[2] Yeditepe University, Faculty of Engineering, Department of Materials Science and Nanotechnology Engineering, İstanbul, Turkey, (ORCID: 0000-0002-1134-1543), ayse.dulda@yeditepe.edu.tr
[3*] Yeditepe University, Faculty of Engineering, Department of Biomedical Engineering, İstanbul, Turkey, (ORCID: 0000-0002-3331-9152), gokhan.ertas@yeditepe.edu.tr

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Abstract

Fluorescence-based optical test strip readers are used to detect and quantify fluorescent signals from immunoassay test strips in medicine, especially for point-of-care applications. The design of optical systems including light sources and detection systems in these devices is not only indispensable but also the most critical part for specific detection applications. This study aims to provide detailed information about fluorescence-based optical test strip readers, existing and emerging technologies, and their contributions to the design of the device. The most commonly used technologies of light sources and detection systems have been discussed and compared for the ideal design. Arc and Xenon lamps may not be appropriate for portable and low-cost devices as they are larger and more costly when compared to LEDs and laser diodes. Photodiodes and CMOS detectors can be used for the design of low-cost, portable fluorescence-based optical test strip readers as they are cheaper and smaller in size when compared to CCDs and PMTs. Both light source and detector should be chosen according to the application priorities and spectral characteristics of the fluorescent molecule by integrating them with proper optical elements like filters, mirrors, etc. This study contributes to the people who are interested in the design of fluorescence-based optical test strip readers as it serves as a guideline for the optical test strip reader systems.

Keywords: Fluorescence, test strip, optical reader, light source, light detector.

Floresan Tabanlı Optik Test Şeridi Okuyucuları için Gelişmekte Olan Teknolojiler

Öz

Floresan tabanlı optik test şeridi okuyucuları, özellikle tıpta immünoassay test şeritlerinden floresan sinyallerini algılamak ve ölçmek için kullanılır. Bu cihazlardaki ışık kaynakları ve algılama sistemlerinin tasarımı sadece vazgeçilmez değil, aynı zamanda en kritik noktadır. Bu çalışma, floresan tabanlı optik test şeridi okuyucuları, mevcut ve gelişmekte olan teknolojiler ve bunların cihazın tasarımına katkıları hakkında ayrıntılı bilgi vermeyi amaçlamaktadır. Optik okuyucunun ideal tasarımı için en yaygın kullanılan ışık kaynakları ve algılama sistemleri tartışılmış ve karşılaştırılmıştır. Ark ve Xenon lambalar, LED'ler ve lazer diyotlara kıyasla daha büyük ve daha maliyetli olduklarından dolayı, taşınabilir ve düşük maliyetli cihazlar için uygun olmayabilirler. Fotodiyotlar ve CMOS detektörler, CCD'ler ve PMT'lere kıyasla daha ucuz ve daha küçük oldukları için düşük maliyetli, taşınabilir test şerit okuyucularının tasarımı için kullanılabilirler. Işık kaynağı ve ışık detektörleri uygulama önceliklerine ve kullanılan floresan molekülün spektral özelliklerine göre, gerekli ise filtreler ile bütünleşmiş olacak şekilde seçilmelidir. Bu çalışma, optik test şeridi okuyucu sistemleri için bir rehber niteliğinde olması nedeniyle floresans tabanlı optik test şeridi okuyucularının tasarımı ile ilgilenen kişilere katkı sağlamaktadır.

Anahtar Kelimeler: Floresan, test şeridi, optik okuyucu, ışık kaynağı, ışık detektörü.

1. Introduction

Fluorescence-based optical test strip readers sense and quantify fluorescent signals from immunoassay test strips in rapid and accurate detection of target analytes at the point of care and are used for environmental monitoring, food safety [1,2], and medical diagnosis [3-8]. These devices employ fluorescence technology in detecting specific substances in a biological sample. The biological sample including a fluorescent molecule, a biological marker, or a fluorophore, is exposed to an excitation light with a specific wavelength (excitation wavelength) that causes target molecules in the sample to fluoresce depending on the spectral characteristics [9, 10]. An excited molecule emits fluorescence light with a specific wavelength (emission wavelength). The light emitted is sensed and processed to determine the presence or absence of a specific substance in the sample. A reader device typically consists of a light source, a light detector, and some optical components such as filters, and mirrors [11-13]. In this work, we provide a brief guide for the design of fluorescence-based optical test strip readers focused on the working principle, benefits, and limitations. In addition, we also discuss the details of fluorescent dyes, light sources, detectors, complementary electronic components, and their integration.

1.1. Working Principle

In a fluorescence-based optical test strip reader device, a sample is placed onto the immunoassay test strip where an interaction between the sample and the reagents takes place. This reaction produces a fluorescence signal or light that is detected by the optical system of the device. Specific wavelengths of the generated light are filtered by positioning optical components such as optical filters, mirrors, etc. [14, 15]. The idea behind fluorescent-based OTSRs is that they can detect fluorescent signals from samples that have been dyed fluorescently. These systems make use of lateral flow immunoassay test strips that house fluorescence dyes, antibodies, and reagents to deliver fluorescence emission quantifying a particular antigen or antibody in the sample [16] (Figure 1 shows a typical physical layout of a lateral flow immunoassay test strip).

A light source within the device generates light emission at a certain wavelength that excites the fluorescent dye in the sample. A detector in the device senses the light that the dye or the marker emits. Correct sensing is very important to quantify the target molecule as the amount of the target analyte in the sample immediately correlates with the produced light intensity [17-19]. In some cases, the target analyte concentration in the sample is estimated by comparing the intensity of the fluorescent signal to a reference signal [20]. Additional components such as a microcontroller, a user interface, and software for data analysis and interpretation are included in the design of optical test-strip reader devices [21]. Some devices are capable of performing several tests at once or storing and recalling test results for further analysis. The devices are commonly used for medical monitoring applications for measurements of analytes such as hormones, as well as point-of-care testing of various infections [22-24] and diseases like HIV [25], malaria [26], and diabetes [27].

1.2. Benefits

Fluorescence-based optical test strip reader devices provide several benefits, including rapid response, accuracy, sensitivity, ease of use, adaptability, and portability. Fast results allow medical practitioners to immediately analyze, and evaluate patient conditions and apply appropriate treatments to the patients earlier. Increased accuracy and specificity reduce the possibility of false positive or false negative findings. The devices can be operated by non-technical individuals. They are small in size and lightweight so they can be easily moved to the point of care. Fluorescence detection is a highly sensitive method for detecting low levels of analytes in the sample for a wide range of applications when compared to other detection methods, namely colorimetric or electrochemical. Furthermore, the method is highly selective for the target analyte, so it can identify and distinguish between different analytes even in complex sample matrices, which contributes to its enhanced use in health [28-30].

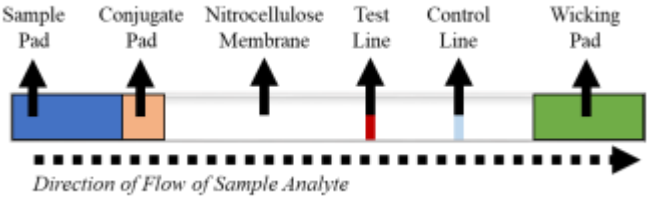

Figure 1. The physical layout of a lateral flow immunoassay test strip

1.3. Limitations

Fluorescence-based optical test strip reader devices suffer from high costs, limited wavelength range, unwanted interference, and the need for maintenance. Depending on the components included in the design, fluorescence-based optical test strip readers can be expensive. The devices may have a limited wavelength range for detection, which may limit the range of fluorescent probes or labels that can be utilized. Detections may be subject to interference from background fluorescence, autofluorescence of the sample components, and other sources of optical noise. This decreases the signal-to-noise ratio and limits the sensitivity and accuracy of the measurements. The maintenance of these optical readers can be challenging as they may require more frequent maintenance than other detection methods, including cleaning, calibration, and replacement of components like optical filters within the device. These limitations can be overcome by using emerging technologies such as optical filters produced using recent technologies [31-33].

2. Material and Method

2.1. Selection of the Fluorescent Dye

Fluorescent dyes, also known as fluorophores, are molecules that absorb light at a specific wavelength and then emit some light at a longer wavelength. There are many different types of fluorescent dyes. Rhodamine dyes (Rhodamine B, excitation/emission wavelength: 554/570 nm) which are known for their excessive illumination [34,35] and photostability [36,37] are preferred for medical imaging including fluorescence microscopy [38], flow cytometry [39] as well as diagnostic tests [40]. Cyanine dyes (Cy5, excitation/emission wavelength: 649/670 nm) are highly sensitive to changes in environmental conditions, such as changes in pH value or the presence of specific molecules, and therefore are often used in biological imaging, including live-cell imaging, and fluorescence resonance energy transfer assays [41-43]. Fluorescein dyes (excitation/emission wavelength: ~495/515 nm) are commonly preferred in optical

readers, to visualize specific biomolecules, such as proteins or nucleic acids, within a biological sample like blood, urine, etc. They are often conjugated to antibodies or other biomolecules to create fluorescent probes that can bind to specific targets in a biological sample to increase the specificity of the diagnosis. The choice of fluorescent dye depends on the application.

2.2. Design of the Optical System

A fluorescence-based optical reader device consists of a light source, some optical filters, a light detector, a sample holder, an electronic control unit, and a power supply unit. These components can be selected and integrated in different ways depending on the spectral characteristics of the fluorescent material considered [44]. Figure 2 shows the block diagram of a typical reader device.

2.2.1. Light Source

The light source plays a critical role in providing the required energy for light emission by the target molecule excited in the sample kept within the lateral flow immunoassay test strip. Common light sources are light-emitting diodes [18, 45-47] and laser diodes [48, 49]. A light-emitting diode (LED) is a solid-state, stable, and compact source producing light when a current is applied. It can be operated with low power while generating a narrow spectral range, so it is very preferable for fluorescence-based OTSRs [46]. They can facilitate the production of specific wavelength ranges to match the excitation and absorption properties of different fluorescent dyes. The possible problem with this type of light source is that commercially available LEDs are produced in narrow wavelength ranges as listed in Table 1. Although a LED can be produced to operate specific wavelengths according to the needs, the cost of production will significantly increase the cost of the reader.

Laser diodes are solid-state semiconductor devices producing highly focused and intense light. Due to their small size, high power efficiency, and ability to produce light with a specific wavelength range from the ultraviolet region (F2 excimer 157 nm) to the mid-infrared region (CO2 10.6 μm) of the spectrum, they are ideal for fluorescence-based optical test strip readers. Three main types of lasers that are gas, liquid (dye), and solid-state-based technologies can be used in specific wavelengths. They can be tuned to various wavelengths by changing the applied current, temperature, or magnetic field, but they are very expensive, which makes optical readers costly. Arc lamps are broad-spectrum light sources generating light by passing an electric current through a gas-filled container. Broad spectrum light generation by these lamps makes optical filters obligatory in optical systems, which causes higher costs for optical readers. Mercury arc lamps providing intense light in the visible range, are used in such reader devices, but additional power consumption, short lifetime, and filter necessity problems are faced. These lamps have illumination lights at different wavelengths based on their broad spectral ranges. 100 Watt mercury arc lamp is one of the types of mercury arc lamps that has unequal light emissions at nine different central wavelengths of 254, 300, 312, 334, 365, 405, 436, 546, and 579 nm. Therefore, it requires an optical filter to emit a specific wavelength of light based on the fluorescent molecule. Xenon lamps are also broad-spectrum light lamps generating light by passing an electric current through a xenon-filled container. Xenon arc lamps of 75 watts have the major light emission at wavelengths in the infrared region of the spectrum (827, 885, 919, 980, 992 nm) while the visible light (475 nm) is provided by only

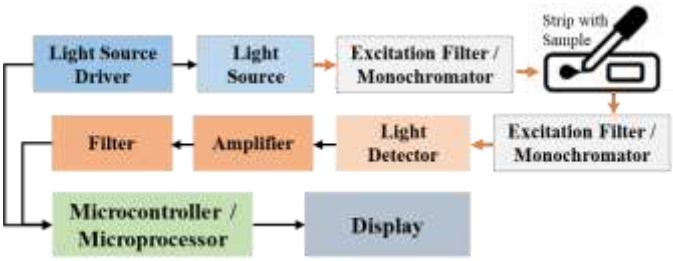

Figure 2. Block diagram of an optical reader device

Table 1. Commercial LEDs

Wavelength (nm)	Spectrum	
410 - 420	Visible (Violet)	Skin therapy
430 - 470	Visible (Blue)	Dental curing
520 - 530	Visible (Green)	Skin rejuvenation
580 - 590	Visible (Amber)	Acne treatment
630 - 640	Visible (Red)	Wound healing
660	Visible (Red)	Blood oximetry
680	Visible (Red)	Blood analysis
800 - 850	Near-Infrared	Pain management
850 - 940	Near-Infrared	Muscle recovery
940	Near-Infrared	Injury recovery

25% with unequal light intensities. Xenon arc lamps are very similar to mercury arc lamps technology, but they provide a more stable and uniform light output even though they are more expensive. These arc lamps are two popular types of lamps used in wide-field fluorescence microscopes.

Table 2 lists the advantages, disadvantages, and application areas of the light sources mentioned above. In some cases, multiple light sources can be positioned in the optical system to detect different fluorescent molecules within the sample. A well-selected light source and a well-defined excitation procedure are needed to increase the accuracy and decrease interference, cost, and size of a reader device. The light source has a significant impact on the quality of the fluorescence signal and should be selected based on the aimed excitation light intensity, and spectral characteristics of the target element.

2.2.2. Optical Filters, Lenses, and Mirrors

The optical filters, lenses, and mirrors, are used to control the light path and direct the excitation light, and produced a fluorescence signal to the detector. These elements are also utilized to decrease the impact of background light and to increase the signal-to-noise ratio. Filtering elements are passive and preferred to improve the reliability level of fluorescence-based optical reader devices. Utilization of filtering elements provides allowing a specific band of light energy to pass through to excite the sample while blocking all other remaining wavelengths. Short-pass, long-pass, and band-pass filters are the three main types of optical filters. Short-pass filters prevent longer wavelengths to be transmitted while enabling wavelengths lower than the cut-off wavelength to pass through. Long-pass filters allow the passing of longer wavelengths and block the shorter wavelengths than the cut-off value. Band-pass filters transmit a

Table 2. Comparison of light sources

Light Source	Advantage	Disadvantage	Place of Use
LED	Cost-effective Compact and stable Low power consumption Broad spectral range Long lifespan	Low light intensity Limited spectral range	General purpose OTSR Portable OTSR
Laser Diode	High light intensity Small size High power efficiency Narrow wavelength range	High Cost Requires cooling Limited spectral range	High sensitivity OTSR Laboratory-based OTSR
Arc Lamp	High light intensity Broad spectral range Wide availability	Large size High power consumption Short lifetime Requires cooling	Laboratory-based OTSR Large-scale OTSR
Xenon Lamp	High light intensity Broad spectral range Stable and uniform light	Large size High power consumption Short lifetime Requires cooling	Laboratory-based OTSR Large-scale OTSR

determined band/range of wavelengths while blocking the others. Also, a monochromator which is a narrow-band example of band-pass filters may be utilized for the filtering aim and its setting should be suitable for the absorption and emission wavelengths of the fluorescent material that is used in the experiment. It is generally used in spectrometers, but may not be affordable for cost-effective optical test strip readers. Also, a dichroic filter/ mirror can be utilized in the design of an optical reader device as it allows the passing of light at specific wavelengths while blocking all the other wavelengths. This mirror reflects specifically defined wavelengths to ensure the light path in optical systems. The filters that can be produced with different properties like wavelength, size, thickness, etc., are used for both excitation and emission light filtering purposes. Therefore, these filters are selected according to the specific wavelength which is planned to be necessary for the spectral characteristics of the target molecule. If the light spectrum of the source and fluorescence is adjusted specifically in the design, filtering elements may be optional in the instrumentation to minimize the cost of the reader device except for broad-range light sources like arc lamps.

2.2.3. Light Detector

The fluorescence light passing through the emission filter is detected by a selected detection system. For a fluorescence-based optical reader design, a detector is crucial as it is used to receive the fluorescence signal which is the main criterion of the analysis to be evaluated. In the detector, the fluorescence light intensity, which is directly proportional to the targeted material concentration, is provided as a digital readout. There are different types of detectors such as photodiodes, photomultiplier tubes, charge-coupled devices, complementary metal-oxide semiconductors, avalanche photodiodes [50], visible light detectors, and spectrometers. However, photodiodes, photomultiplier tubes, charge-coupled devices, and complementary metal-oxide semiconductors are preferred commonly [51, 52].

Photodiodes are simple photodetectors that convert light into electrical signals and are widely used in optical test strip readers due to their low cost and ease of use [53]. Photomultiplier tubes (PMTs) are highly sensitive photodetectors that amplify the fluorescence signal using a cascade of dynodes and are capable of detecting very low-intensity light, making them very applicable for highly sensitive fluorescence light occurrences. [54] They are more expensive than single photodiode detectors. Charge-coupled devices (CDDs) are known as solid-state photodetectors that detect fluorescence signals by converting light into electrical charges and are ideal for imaging applications and are capable of producing fluorescence signals with great resolution and sensitivity in a high dynamic range [55]. They are widely used in the case of image-based analysis in optical systems despite their cost and maintenance problems. Complementary Metal-Oxide Semiconductor (CMOS) sensors are solid-state photodetectors that enable detecting fluorescence signals by converting the light into electrical charges and are well-suited for low-cost, low-power fluorescence measurements and are commonly preferred in consumer-based electronics [45]. Avalanche photodiodes (APDs) are photodetectors that apply an internal gain mechanism to amplify low-intensity fluorescence signals making them ideal for highly sensitive fluorescence measurements. There are also single-photon avalanche diodes that are capable of detecting individual photons in highly sensitive fluorescence signal measurements for optical reader devices.

Table 3 lists the advantages and disadvantages of the light detectors mentioned above. The detection range of the system can be adjusted for the detection of different molecules by the inclusion of optical filters positioned in front of the detector, or detection systems with broader detection ranges are redesigned with these filters to increase the accuracy and reliability of the measurements. The choice of the detection system depends on the specific requirements of the device, including sensitivity, specificity, cost, size, and compatibility with the used fluorescence dyes.

Table 3. Comparison of light detectors

Light Detector	Advantage	Disadvantage	Place of Use
Photodiode	Low cost Simple design Widely available	Low sensitivity Low dynamic range Affected by electronic noise	Portable OTSR Low-cost OTSR
Photomultiplier tube	High sensitivity High dynamic range Capable of detecting very low light levels	Large size High cost Require high power Sensitive to electromagnetic interference	Laboratory-based OTSR Large scale OTSR
Charge-coupled device	High sensitivity High dynamic range Capable of imaging fluorescence signals Solid-state design	Large size, high cost Electronic noise Sensitive to electromagnetic interference	Imaging OTSR High-resolution OTSR
Complementary Metal-Oxide Semiconductor	Low cost Low power consumption Solid-state design Widely available	Low sensitivity Low dynamic range compared to PMTs and CCDs Affected by electronic noise	Portable OTSR Low-cost OTSR Consumer OTSR
Avalanche photodiode	High sensitivity High dynamic range Capable of detecting low light intensity Fast response time	High cost Require high power Sensitive to electromagnetic interference Open to thermal noise	Laboratory-based OTSR Large-scale OTSR

2.2.4. Complementary Electronic Components

Additional electronic components such as buttons and displays can be included in the design of these optical reader devices to improve the simplicity and ease of use for user interface and experience. Their design must be optimized to ensure that the optical test strip reader is accessible and usable for a wide range of users, including patients, healthcare professionals, and laboratory technicians [56]. The algorithm and software must be developed to analyze the obtained fluorescence signal generated by the fluorescent molecules in sample holders like test strips and to process this data to generate appropriate results. The program must be user-friendly with an easy-to-understand interface, and be capable of accurately and rapidly processing massive data.

3. Results and Discussion

In fluorescence-based optical test strip readers design, different existing fluorescence emitting elements like fluorescent dyes, detection antibodies, and other reagents can be used to achieve fluorescence generation. Among them, fluorescent dyes/fluorophores including Rhodamine, Cyanine, and Fluorescein dyes are commonly used as they provide specific detection opportunities by absorbing and emitting light at certain wavelengths. There are recent technologies utilizing these dyes for the production of fluorescent bioprobes for medical and biological imaging in medical diagnostics.

The main component of a reader device is the light source for generating the excitation light and the light detector for sensing the fluorescence light. Different technologies of light sources such as LED, Laser, Arc Lamp, and Xenon lamp, can be utilized. LEDs provide more stable light in a narrow spectral range while consuming low power for the operation that lasts longer. They are cheaper when compared to other light sources, but existing commercially available LEDs have certain bands of wavelengths (410-940 nm). Also, the light intensity of LED sources is lower than that of laser diodes. Laser diodes provide focused, intense light in both visible and infrared ranges with high power efficiency. They can be found in specific wavelengths although they are more expensive and needs additional cooling. Moreover, Arc and Xenon lamps are similar technologies providing high light intensities in a broad spectral range. Both types of lamps require specifically adjusted optical filters, larger spaces to be placed, and need more power for the operation. Xenon lamps differ from arc lamps as they provide more stable and uniform light.

Photodiodes, PMTs, CCDs, and CMOS are the most common detection technologies for use in optical readers. Photodiodes are widely available for use in optical readers since they are affordable prices. However, their response is less sensitive to light intensity and operates at lower dynamic ranges while being affected by electronic noise. The sensitivity property is much more enhanced in PMT technologies in great dynamic ranges, but those require higher costs, increased space, and more power to operate. CCDs offer the same advantages and additional imaging capabilities whereas they can be easily affected by electromagnetic interference. As another option, CMOS devices can be used as a detection system in reader devices with lower costs, but their dynamic range and sensitivity level are less than PMTs and CCDs. These drawbacks can be handled by using APDs while more costs and power consumption problems are likely to

be confronted. In the optical system, some additional elements like optical filters, mirrors, and lenses can be used to direct both excitation and emission light. Optical filters are adjusted based on the spectral characteristics of the fluorescent sample to be detected. Based on their properties, optical filters have three main types: Short-pass filters allowing shorter wavelengths to pass, Long-pass filters allowing longer wavelengths to pass, and Band-pass filters allowing a range of wavelengths to pass through the light path. For different light directions and detection purposes, these filters can be designed and created. A dichroic mirror can also be used to reflect light with specific wavelengths. All of the optical filters can be produced with different properties (like wavelength, size, thickness, etc.) with emerging technologies to increase the specificity and reliability of the optical device. Short-pass, Long-pass, and Band-pass) optical filters and dichroic mirrors can be used in fluorescence-based OTSRs. Monochromator filtering element which is more expensive can also be thought of as an alternative for the same purpose in fluorescence-based optical test strip readers.

Fluorescence-based optical test strip readers provide several benefits, but they also have certain drawbacks that should be taken into account when choosing a detection strategy for a specific application. All of these properties depend on the instrument design by selecting appropriate components to achieve an applicable and more effective diagnostic reader device. In medical applications, fluorescent dyes are often conjugated with antibodies or other biomolecules to create fluorescent probes that can bind to specific targets in a biological sample to increase the specificity of the diagnostic device. The preparation of these materials involves optimizing their stability, specificity, and sensitivity for use in the optical test strip reader. There are different types of fluorescent dyes. Among them, fluorescein dyes are beneficial for medical imaging and diagnosis, as they facilitate the detection and visualization of specific biomolecules within a biological sample. Fluorescent dye selection depends on the application priorities and the features of the optical system.

LEDs, laser diodes, arc lamps, or xenon lamps, are used as light sources in optical test strip readers. LEDs provide more stable light in a narrow spectral range while consuming low power for the operation that lasts longer than laser diodes. They can be placed in the design of cost-effective optical readers. Although they are cheaper when compared to other light sources, commercially available LEDs have certain bands of wavelengths. That means a higher budget is needed to develop LEDs in specific wavelengths. In contrast, a laser diode of a specific wavelength can be obtained easily. They can be used especially for portable fluorescence-based optical readers in which more intense excitation light is needed. Both LEDs and laser diodes are small in size as well as require less power, making them suitable for portable fluorescence-based optical test strip reader devices. Another alternative is using arc or xenon lamps in the optical reader system. They may cause more costly reader device designs with a shorter lifetime when compared to LEDs and laser diodes, so there can be some battery/charge problems for the devices. In addition to this, they are larger, so they may not be the correct choice for the portable design of an optical reader. An important difference between these lamps is that a more stable and uniform light can be achieved by the usage of xenon lamps instead of arc lamps. The selection of a light source is affected by the precise needs of the optical test strip reader, such as the desired sensitivity and specificity, the financial and physical limitations, and the compatibility with the fluorescence dyes utilized. In fluorescence-based optical test strip readers, both LED and laser diodes are frequently used as light sources, so the best option can be chosen based on the device's unique specifications.

Short-pass, long-pass, and band-pass optical filters are constructed based on their filtering principle. The correct optical filter should be selected according to the optical components like the light source, the detector, and the fluorescence-emitting material in the fluorescence-based OTSRs. For specific wavelength adjustments, a monochromator element can be placed in the light detection system, but this element is more expensive than the other filter types. A dichroic mirror can be placed to reflect the light in a wavelength range while eliminating others. These components are to maximize the signal-to-noise ratio, although they all increase the cost of the device.

The light detectors are selected regarding the purpose of use of the reader device under development. For cost-effective readers, Photodiodes or CMOS devices can be utilized, but both of them have sensitivity issues and lower dynamic ranges for a variety of measurements. PMTs, CCDs, and APDs are highly sensitive to greater dynamic ranges compared to other options. Between them, APDs provide rapid responses during analysis while PMTs can detect very low levels of light intensity. Also, CCDs are large, so they may not be suitable for portable diagnostic reader devices in general. As seen from these results, each detection technology can provide separate properties according to their usage aim. Therefore, the choice of the detection system is dependent on the specific requirements of the device, including sensitivity, specificity, cost, size, and compatibility with the fluorescence dyes used.

In this study, fluorescence-based optical test strip readers, emerging and existing technologies, their advantages and disadvantages, and contributions to the device design with their remarkable properties are discussed. This study explains, compares, and discusses different technologies for optical system design in such diagnostic readers. For these reasons, this paper will contribute to people who are interested in the practical design of fluorescence-based optical test strip readers by serving as a guideline for the design of an optical system for fluorescence-based test strip readers with emerging technologies.

4. Conclusions and Recommendations

Diagnostic fluorescence-based optical test strip readers are widely used to detect and quantify fluorescent signals from immunoassay test strips, especially in the medical area for point-of-care applications. It offers many advantages like fast readings, accuracy, portability, ease of use, sensitivity, versatility, and selectivity whereas it can be affected by interference effects in limited wavelength ranges requiring frequent maintenance. Fluorescein dyes are valuable markers for medical imaging and diagnosis, as they facilitate the detection and visualization of specific biomolecules within the test strip inserted into the reader device. Two critical issues for fluorescence-based optical test strip reader design are the light source and the light detector. For light sources, LED, laser Diode, arc lamp, and xenon lamp technologies can be utilized in the optical system. For cost-effective devices, LED light sources are suitable for excitation purpose but their light intensity is lower than the laser diodes which costs higher and needs cooling. Moreover, Arc and Xenon lamps are similar technologies providing high light intensities in broad spectral ranges, but they may not be appropriate for portable and low-cost devices as they are larger and more costly when

compared to LEDs and laser diodes. Short-pass, long-pass, and band-pass filters are the three main types of optical filters that are classified based on their filtering principle. The type of filters and mirrors should be selected according to the spectral properties of the light source, detector, and fluorescent material in the fluorescence-based optical test strip readers. For the design of the detection part, photodiodes, PMTs, CCD and CMOS are the most common detection technologies for use in optical readers. Photodiodes and CMOS devices can be used for the design of low-cost fluorescence-based optical test strip readers. Other mentioned technologies, PMTs, CCDs, and APDs, are highly sensitive to greater dynamic ranges compared to photodiodes and CMOS. Furthermore, CCDs are large, so they may not be suitable for portable readers. In the optical system, some additional components can also be utilized to achieve more reliability and specificity of the device. All of the optical components should be adjusted based on the spectral characteristics of the fluorescent molecule to be detected in the sample. These can be implemented using different methods with emerging technologies. The light sources and detection systems must be selected to maximize the sensitivity and specificity while minimizing the cost and the size of the reader device.

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