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Could Femtosecond Laser-Induced Fluorescence Be a Potential Hopeful Strategy for Rapid Monitoring of Serum Albumin and Cardiac Troponin?

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Abstract

Blood analysis of various biomarkers is usually required in medical diagnosis essential for disease detection and health status monitoring. Human serum albumin (HSA) is the most abundant serum protein, indicator of liver and kidney diseases. A protein member of the cardiac troponin complex called Cardiac troponin 1 (cTn1) is used to diagnose several pathologies associated with cardiomyocyte necrosis. Laser-induced fluorescence (LIF) is a spontaneous emission of atoms or molecules that have been excited by laser radiation. LIF is one of the most significant developments used as an analytical tool for qualitative and quantitative analysis is a technique with high sensitivity and specificity. LIF is one of the most important advancements utilized as an analytical technique for qualitative and quantitative assessment because it offers high sensitivity and specificity. This article introduces the potential hopeful strategy of femtosecond

LIF in clinical analysis as a new detection method for monitoring certain serum biomarkers such as HSA and cTn1. The femtosecond LIF offers a novel, highly sensitive, accurate, and direct technique for monitoring the HSA and cTn1.

Keywords: Biomarkers; Human serum albumin (HSA); Cardiac troponin 1 (cTn1); Laser-Induced Fluorescence (LIF); Femtosecond laser.

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1. Introduction

Utilizing precise clinical assessment methods to track disease progression and the outcomes of interventions (vaccines, drugs, surgery, etc.) is one strategy for conducting therapeutic research more quickly and effectively (A A Alizadeh 1, 2000). Another strategy is utilizing a variety of analytical tools to evaluate biological factors, often known as biomarkers (Rolan, 1997).

A biological marker (biomarker) is a characteristic that can be quantitatively measured and evaluated to indicate pathogenic processes, healthy biological processes, or pharmacologic effects on treatment intervention. Essential uses for biomarkers are disease detection and health status monitoring. Additionally, biomarkers have a variety of uses, such as a diagnostic method for determining which people with the disease or an abnormal condition, used to determine and evaluate the clinical response of an intervention, and used as an indicator for the prognosis of diseases (Atkinson, 2001). An ideal biomarker is typically anticipated to be: accurate, capable of detecting a major feature of a particular disease, and able to differentiate between cases of this specific disease in its early stages and those of its relatives or other similar diseases simple, reliable, non-invasive, and if at all possible inexpensive procedures (Ji Gao a 1, 2005; Ronald & Aging, 1998).

The main advantages of biomarker analysis are its speed, accessibility, and affordability because it is possible to use both point-of-care portable devices (POC) and a research lab setting (Mengxing Ouyang 1, 2021). As a result, medical diagnosis usually requires blood analysis (blood biomarkers) (Annika M K Enejder, 2002). The essential tool for disease diagnosis is the quick and accurate detection of protein imbalances in serum (Hardy & Selkoe, 2002; Pulido & Hooft van Huijsduijnen, 2008). The serum contains 60-80 mg/ml of proteins (Ikemi & Ikemi, 1986). More than 95% of the total serum proteins are made up of IgA, haptoglobin, albumin, α1-antitrypsin, fibrinogen, α2-Macroglobulin, complement C3, and transferrin (Sahab et al., 2007). There are 20,000 distinct proteins present, ranging from 50 g/l (serum albumin) (Adkins et al., 2002; Pieper et al., 2003) to less than 1 ng/l (troponin) (Antman et al., 1996), for a total protein concentration of 1 mM. Specific disease states are directly correlated with the relative and absolute levels of these proteins (De et al., 2009).

2. Proteins

Proteins are the primary constituents of blood serum or plasma, they are macromolecules made of one or more unbranched chains of amino acids linked by peptide bonds (Stockham Steven & Scott, 2002). Approximately 20 amino acids constitute proteins (Roger L. Bertholf, 2014). Hepatocytes generate and secrete almost all serum proteins (Eckersall, 2008). Proteins serve a variety of functions, almost every reaction that takes place within an organism involves proteins, including those that catalyze metabolic reactions (enzymes), proteins that maintain the colloid osmotic structure, and buffer acid-base equilibrium. Other proteins play a crucial role in the control of cellular activity and the immune system (Anderson & Anderson, 2002), in platelet adhesion and aggregation (Meyer & Harvey, 2004), transducing chemical signals (cell-surface receptors), and carrying vital ions, in an exocrine and the endocrine system such as (insulin, thyrotropin, and gonadotropins), and in preserving intravascular and interstitial fluids' osmotic balance (Human serum albumin; HSA) (Roger L. Bertholf, 2014).

2.1. Human serum albumin

The HSA is the most abundant protein in the blood (Peters, 1985), is the essential protein in the circulatory system (Lin et al., 2020), and makes up 60% of the blood serum total protein composition (Guizado, 2014). HSA has low molecular weight (66,5 KDa), and is a single-chain protein that has 585 amino acids (Raoufinia et al., 2016). HSA is a tiny globular protein that is highly water-soluble, HSA levels range from 3.5–5 g/dl (Larsen et al., 2016). The HSA structure is shown in Fig. 1 (Abou-Zied & Sulaiman, 2014).

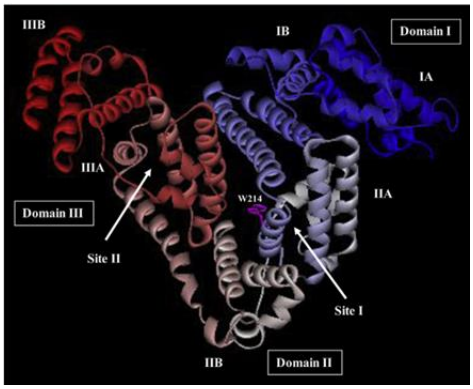

Fig. 1. The crystal structure of HSA and the locations of domain-binding sites. The locations of hydrophobic binding sites (Site I and Site II) are shown. The position of tryptophan residue (warfarin site (W214)) in subdomain IIA is shown (Abou-Zied & Sulaiman, 2014).

The hepatocytes produce albumin, but the liver doesn't keep it, it is secreted into the portal circulation when it is produced (Quinlan et al., 2005) and then released into the bloodstream (Moman & Varacallo, 2018). The rate of HSA synthesis in the liver is 9–12 g/day (Caraceni et al., 2013) and HSA a biological half-life of 19 days in humans (George & Erasmus, 2018). The HSA has many functions, it serves several crucial roles in preserving blood PH (Chilom et al., 2020) and maintaining the plasma's colloid osmotic pressure (Bomholt et al., 2021).

Albumin has a great affinity for sodium ions as well as other cations, water is drawn into the blood vessels as a result of albumin's attraction to sodium (1 g of albumin may retain 18 ml of water inside the blood vessels) (Ge et al., 2016). Additionally, HSA serves as a vital transport protein (Lin et al., 2020), it acts as

a binding site and carrier for endogenous and exogenous (Arroyo et al., 2014; Roohk & Zaidi, 2008). HSA frequently decreases and offers reliable and powerful information about the prognosis of diseases (Ronit et al., 2020), it is a crucial biomarker used in clinical settings to assess liver function (Rabbani & Ahn, 2021).

2.1.1. Human serum albumin clinical significance

Albumin synthesis is controlled by a variety of variables, including nutritional conditions, oncotic pressure, and hormonal factors (Rabbani et al., 2018). Reduced gene transcription and ribosome disaggregation can alter albumin metabolism, resulting in its low synthesis (Bertucci & Domenici, 2002). This leads to a drop in albumin levels, protein breakdown may increase, protein loss through the kidneys may increase, and the volume of the liquid component of blood (plasma) may increase, thinning the blood (Ge et al., 2016). Hypo-albuminemia is a common sign of the disease that is defined as an insufficient decrease in HSA concentration in the blood. This is not simply due to a decrease in albumin synthesis; it is also influenced by protein uptake, degradation, and leaking into the extravascular space (Ballmer, 2001; Lee et al., 2014). In addition to chemotherapy, infections, cancer, liver and kidney disease, AIDS, surgery, burns, several medications, and respiratory diseases are examples (Eljaiek & Dubois, 2013; Finfer et al., 2006; Fritz et al., 2003; Kobayashi et al., 2004; Ryan et al., 2007; Vlahos et al., 2005). Serious liver disease and kidney disease can change albumin concentration. Since the liver produces albumin, a decrease in liver function during conditions such as chronic hepatitis, cirrhosis, or liver failure might cause albumin levels to drop. Maintaining albumin and avoiding its elimination in urine with waste materials is one of the kidneys' primary functions, the ability of the kidneys to maintain plasma protein concentrations is lost when they get damaged in conditions like diabetes, and hypertension (Ge et al., 2016; Lee, 2012).

The low albumin concentrations may also be a sign of chronic malnutrition, and an insufficient protein diet or they may be linked to parasitic infections, gastrointestinal disorders, and protein-losing enteropathies (Diogenes & Suassuna, 2010; Don & Kaysen, 2004). Hyperalbuminemia, or an elevated level

of albumin in the serum, can occur when someone is severely dehydrated (Cooper et al., 2009). Circulating serum albumin levels that are either too high or too low are hazardous to human health. For instance, an abundance of albumin causes renal illness, whereas a shortage of albumin causes edema. Therefore, accurate methods for measuring the HSA concentration in human serum are crucial for making diagnoses of various diseases (Yang et al., 2018).

2.1.2. Human serum albumin detection methods

Numerous analytical methods for determining the HSA level are available, which will be discussed as follows (Chen et al., 2017).

A. Dye-binding techniques

Several dye-binding techniques for measuring albumin were created to make it possible to estimate HSA quickly and accurately (Doumas & Peters, 2009). The most popular assay that includes dye-binding is Bromocresol green (BCG) (D. Kumar & D. Banerjee, 2017). Bromocresol green (BCG) is a phthalein dye that Rodkey invented in 1964 for measuring albumin calorimetrically (Boyer, 2000; Gornall et al., 1949; P. G. Hill, 1985; Peters Jr, 1995). Later, the method was enhanced to improve the specificity of BCG for HSA (Peters Jr, 1995). Albumin binds quantitatively to bromocresol green to generate a strong blue-green complex (Kessler et al., 1997), that can be detected spectrophotometrically at 625 nm (Peters Jr, 1995). Although quick, inexpensive, and simple to use, the BCG approach is less sensitive and selective than immunoassays (Doumas & Peters, 1997). The BCG approach overestimates albumin due to its high reactivity with α2- macroglobulin and is not recommended for use in cases of nephrotic syndrome (Ueno et al., 2016).

B. Immunological /immunochemical methods

The HSA concentration can be measured quantitatively in serum and other bodily fluids (such as urine) using immunochemical techniques. The process by which albumin is detected immunochemically is known as albumin/anti-albumin antibody complex formation. Human albumin can be identified with excellent specificity thanks to the availability of monoclonal anti-HSA antibodies. Anti-HSA antibodies increased the sensitivity of albumin detection when combined

with fluorescent probes or standard enzymes for enzyme-linked immunosorbent assay (ELISA) (Spencer & Price, 1979).

There are two types of immunoassays noncompetitive (Sandwich ELISA) and competitive ELISA, both are frequently used to determine the albumin concentration in the sample (Ide & Akani, 2011). Noncompetitive involves the binding of two antibodies to antigenic sites (epitopes) that do not share common ground on the antigen (Mennink-Kersten et al., 2004). Noncompetitive use an abundance of antibodies to capture all of the target antigens present (Roger L Bertholf, 2014). In a competitive ELISA, the antibody in the coated well competes with albumin, which includes the predetermined quantity of albumin labeled with an enzyme and the specimen. This competition decreases the binding of enzyme-labeled albumin (D. Kumar & D. J. C. C. A. Banerjee, 2017). Antigens are abundant in competitive immunoassays, and labeled antigens compete with endogenous antigens for binding sites on a limited number of antibodies (Roger L Bertholf, 2014). In general, ELISA yields quick results and only requires a small number of anti-albumin antibodies, whereas is less albumin-specific (Choi et al., 2004; Ide & Akani, 2011; Tsai et al., 2016; Watts et al., 1986). ELISA has great sensitivity and specificity for simultaneously analyzing numerous markers (Visintin et al., 2008) but it is a complicated procedure and expensive (Wang et al., 2011).

In Radioimmunoassay (RIA), for antibody-binding locations, the specific quantity of radioactively labeled albumin (125I-albumin) and albumin from the test sample are competing in a liquid phase. The sample's albumin concentration is calculated after separating free albumins. Although RIA provides better precision, its use is complicated because of the threats caused by radiation and radiochemistry. As a result, RIA is not widely used in clinical chemistry laboratories to estimate albumin levels (Ide & Akani, 2011; Watts et al., 1986).

In Immunofluorescence, albumin in the sample is specifically bound by fluorescent-labeled anti-albumin antibodies, forming complexes. A flow cytometer or fluorescence scanner will then examine the fluorescent complexes to determine the amount of albumin present in the test sample. This approach is easy, quick, and trustworthy (Choi et al., 2004; Nargessi et al., 1978). This

method is combined with chromatographic separation tools. However, there is currently little clinical experience with this immune-chromatographic method (Choi et al., 2004). In general, Albumin levels may be underestimated by immunochemical approaches because they only quantify immunoreactive albumin and cannot identify fragmented albumin (Brinkman et al., 2004; Redon, 2005). Immunochemical procedures are likely the most precise, but they are also requiring the most funds and workforce (P. J. A. o. c. b. Hill, 1985).

C. Techniques based on electrophoresis

Electrophoresis is utilized to distinguish the sample portions of charged particles depending on how well they move along an electrical gradient, because of the charge difference. It is used for distinguishing the macromolecules and albumin from each other (Pandey et al., 2008). Anti-albumin antibodies are used in the electrophoresis technique, and it was first possible to measure albumin in a gel in 1965. This methodology was indeed rooted in the idea that unbound albumin has different electrophoretic mobility than complexes of antigen and anti-albumin antibodies. This technique for estimating albumin is quick and uses fewer anti-albumin antibodies (Laurell, 1966). Today, gel electrophoresis could be utilized for the identification of both immune-reactive and immuno-unreactive albumin, due to technological developments along with increased gel electrophoresis experience managing clinical samples (Anguizola et al., 2013). The least accurate methods are electrophoretic ones as studies have shown that repeat sample variations can reach 11 % (P. G. Hill, 1985).

2.2. Cardiac troponin 1

The cTn1 is considered a protein biomarker, a protein member, of the cardiac troponin complex (Wu et al., 1998). It was proposed in the 1960s that native tropomyosin, which is found on actin filaments, a unique protein complex, controls the contraction of striated muscle (Ebashi et al., 1968). The main composition of native tropomyosin is troponin and tropomyosin (Bailey, 1946). The troponin complex breaks down during cardiac injury, releasing its protein component such as cTn1, into the blood. (Wu et al., 1998). Three proteins that make up the troponin complex which controls the contraction of skeletal and cardiac muscles are shown in Fig. 2 (Barberi & van den Hondel, 2018), and they

include: Troponin C (TnC) attaches to calcium ions and induces structural changes in TnI. Troponin T (TnT), binds to tropomyosin to form the Tn-tropomyosin complex. Troponin I (TnI) binds to actin and restricts the activity of ATPase. (Filatov et al., 1999; I. Katrukha, 2013; Radha et al., 2021). In humans, troponins 1 and T consist of three isoforms: fast skeletal, slow skeletal, and a unique cardiac isoform (cTn1 and cTnT) (Chaulin, 2021; I. A. Katrukha, 2013). The cTn1 and cTnT have cardio-specific amino acid sequences, making them ideal biomarkers for cardiac injury (Barberi & van den Hondel, 2018). Consequently, cardiac injury can be identified through cTn1 and cTnT, as highly sensitive and specific biomarkers. (Komarova et al., 2022). The cTn1 is "the gold standard" cardiac marker for diagnosing acute myocardial injury (AMI) in clinics due to its outstanding tissue specificity, which guarantees a precise outcome (Guo et al., 2009).

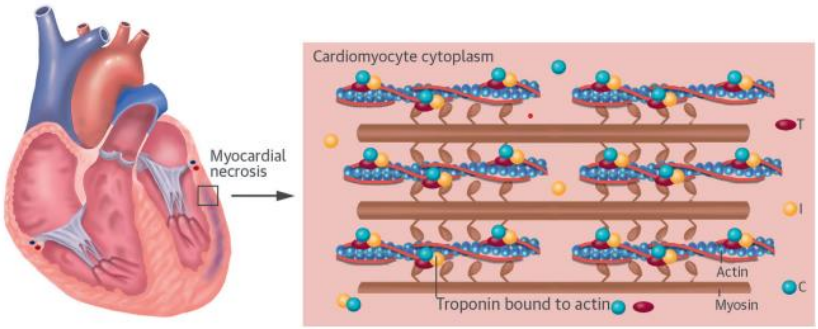

Fig. 2. Troponin complex that is released into the bloodstream when heart tissue is damaged due to acute myocardial infarction (AMI) or other causes (left side). Troponin C, troponin I, and troponin T are three molecules that are either bound to actin or free in the cytoplasm of the cardiomyocyte (right side) (Barberi & van den Hondel, 2018).

The size of cTn1 is a 210-residue-long protein (Cheng & Regnier, 2016; Marston & Zamora, 2020), and its molecular weight 23, 876 KDa (Horak et al., 2015). The main function of cTn1 is to inhibit actomyosin ATPase activity (Leavis et al., 1984) and interact with other components of the thin filament (Perry, 1979, 1999). The risk of heart attack death can be reduced by early detection of cTn1 in the serum of patients with a higher risk of AMI (Daubert & Jeremias, 2010). cTn1 is frequently quantified in emergency departments because of diagnostic,

management, and prognostic implications (Arenja et al., 2012; Celik et al., 2011). An increase in cTn1 concentration was seen in patients who shared all of the same symptoms may call for rapid catheter angiography, hospitalization of the patient, and possible therapeutic intervention as opposed to supportive care treatment and possible same-day discharge (Anderson JL, 2013). Also, cTn1 is one of the most effective heart-specific circulating biomarkers in cardiovascular (CV). Their adoption into clinical practice significantly enhanced the ability to differentiate between different causes of acute chest pain and provided a solid method for diagnosing acute coronary syndromes (ACS) (Jortveit et al., 2022).

2.2.1. Clinical significance and disorder of cardiac troponin 1

The World Health Organization (WHO) forecasts that by 2030 about 23.6 million people will die from cardiovascular diseases (CVDs) (Bozdogan et al., 2020). cTn1 blood analysis is used in the diagnosis of CVDs (Szunerits et al., 2019). cTn1 has been used for the assessment of conditions linked to cardiomyocyte necrosis (myocardial infarction (MI), myocardial trauma, and others) for the last 25 years (I. Katrukha, 2013). Necrosis is the most obvious cause of cTn1 elevation in a patient, particularly if the cTn1 rise is momentary (Hammarsten et al., 2018). In AMI, levels of cTn1 elevated in blood serum (I. A. Katrukha, 2013; Patil et al., 2011), AMI is a significant factor contributing to illness and death globally (Mendis et al., 2015; Smiseth & Tendera, 2008), whose mortality rates rise in the absence of prompt and effective treatment in an emergency. The European Society of Cardiology (ESC) recommends using cTn1, a biomarker protein found only in the myocardium, to rule in or rule out AMI. (Hasić et al., 2003; Roffi et al., 2016). As the molecule is released into the blood when the heart is injured, increased cTn1 levels signify the death of cardiac muscle cells. The distinction between healthy individuals and patients is thought to be made by cTn1 concentrations in the range of 0.5-2 ng/mL (Shan et al., 2014). While the other biomarker will soon fall after an AMI epidemic, cTn1 concentrations can increase to 550 ng/mL and stay elevated for a few days (Engvall & Perlmann, 1971; Shen et al., 2014). cTn1 concentration reaches its highest, and the elevated level lasts for roughly 10 days (Fathil et al., 2015; Han et al., 2016). Patients without cardiac disease have very low or undetectable serum troponin levels (Babuin & Jaffe, 2005). Additionally, cTn1 associated

marker in non-CV conditions such as ACS, cardiotoxicity, kidney disease chronic artery disease, and heart failure (HF), (Cardinale et al., 2018; Farmakis et al., 2018; Kvisvik et al., 2017; McCarthy et al., 2018; Michos et al., 2014; Teerlink et al., 2013), coronary intervention and spasm (Sato et al., 2003), as well as in situations like myopericarditis, septicemia, cardiac trauma, and chemotherapy that result in non-ischemic myocardial injury (Apple et al., 2002)

2.2.2. Methods for detecting cardiac troponin 1

Several different cTn1 detection techniques recently have been applied to monitor cTn1, such as biosensors based on spectroscopy (Han et al., 2016; Qureshi et al., 2012). Optical biosensors include, Colorimetric, Fluorescence, Luminescence, and SPR optical biosensors are among the various types (Fan et al., 2008). The biosensors are made up of the signal processing system as a third component in addition to the two main parts, the recognition element and the transducer (Chambers et al., 2008; Perumal & Hashim, 2014). The transducing structure is critical in optical biosensors such as Grating couplers (Ehrentreich-Förster et al., 2003), Resonant mirrors (Skládal, 1999; Zourob et al., 2009), SPR (Green et al., 2000), or Interferometry. The recognition element captures the target analyte, allowing it to be isolated and thus quantitatively analyzed. Examples of the recognition components used frequently to capture target analytes include antibodies, aptamers, nucleic acids, and enzymes (Regan et al., 2018). The following are detection methods based on biosensors.

2.2.2.1. Immunosensors and Immunoassay

Researchers created immunoassays for cTn1 in the late 1980s (Cummins et al., 1987; Katus, 1991), and used it as an analytical strategy, for quantitative detection based on the reaction of antigen and antibody (Cipok et al., 2016; Ding et al., 2016; Park et al., 2016). The recognition element in an Immunoassay/Immunosensor is an antibody (polyclonal, monoclonal, and recombinant), each with unique properties and production methods. Monoclonal antibodies, which have been found widespread in clinical use as diagnostic and therapeutic agents for several disorders, have been used in a variety of diagnostic tests for the quantitative assessments of cTn1 in human blood (Sanjay et al., 2015). Immunoassays have received a great deal of attention in clinical and

research settings over the last few decades (Melanson et al., 2007). The detecting antibody is linked to the detection process's signaling mechanism and signal amplification (Radha et al., 2021). There have been reports of numerous signaling methods, including optical (colorimetric, fluorescence, and emission), and electrochemical, as well as SPR (Braga et al., 2020; Cho et al., 2014; Han et al., 2016; Haushalter et al., 2016; Ju et al., 2011; Lee & Kang, 2013; Lopez-Calle et al., 2017; Mayilo et al., 2009; Park et al., 2013; Rodenko et al., 2017; Singal et al., 2016; Tan et al., 2017; Tang et al., 2013; Wang et al., 2020; Xu et al., 2020; Zhang et al., 2014; Zhu et al., 2011).

A. Colorimetric assays (chemiluminometric biosensors)

Wu and colleagues proposed a colorimetric biosensor for cTn1 detection in 2010. Calorimetric immunoassays work by measuring the amount of light absorbed by the chromogenic reagent at a specific wavelength to detect the presence of the target analyte. ELISA is the greatest widely utilized and prevalent calorimetric approach. This works on the sandwich immunoassay principle, illustrated in Fig. 3 (Radha et al., 2021), in which the primary antibody binds to the antigen, and then the secondary enzyme-linked antibody binds to it. The amount of enzyme activity that is conjugated by the product is utilized to identify the target analyte (Tayyab et al., 2020). The most frequently employed to develop a colorimetric assay based on this principle is the chemiluminometric signal generated by horseradish peroxidase (HRP). (Wu et al., 2009). While colorimetric measurement is less sensitive, it is easily noticeable and convenient. These techniques generally performed well, however, they have drawbacks in terms of sensitivity, miniaturization, and cost-effectiveness (Homola & Piliarik, 2006).

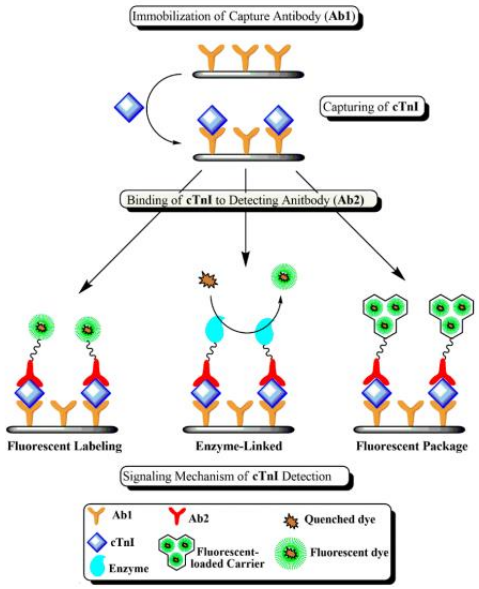

Fig. 3. The sandwich immunoassay design and steps (Radha et al., 2021).

B. Fluorescence-based biosensors

There is an excellent technique that serves as a great alternative to traditional colorimetric devices, fluorescence-based biosensors, which have limitations in terms of their sensitivity and accuracy despite being affordable, straightforward, and quick to read visually. In 1941, Coons et al. developed for the first time a known fluorescence-based biosensor (Coons et al., 1941). The target analyte or component of biorecognition is marked with a fluorescent label, such as a probe or dye. The fluorescence intensity illustrates the target molecule's binding. Although fluorescence-based biosensors are highly sensitive, their main disadvantage is the time-consuming labeling process. Furthermore, controlling the fluorescence signal produced by each fluorophore quantity on each molecule is difficult, rendering quantitative analysis challenging (Cox & Singer, 2004). Fluorescent labels or dyes are used to label the target or biological components in colorimetric or fluorescence detection biosensors (Hasanzadeh et al., 2013). When the intensity of the fluorescence or color signal changes, it indicates the presence of target molecules.

C. Chemiluminescence and electrochemiluminescence

Chemiluminescence and electrochemiluminescence (ECL) biosensing have been evaluated (Hu & Xu, 2010; Jie et al., 2007). The ECL is defined as the generation of luminescence while an electrochemical reaction is occurring. Positive characteristics of ECL, broad linearity, minimal background, elevated sensitivity, and simplicity in detecting acute myocardial infarction biomarkers are considered (Sperling et al., 2008). Most of the methods are categorized as generating electrochemical signals for enhancement (Li et al., 2013). The intensity of electrochemiluminescence was used to calculate the concentration of cTn1 (Zhang et al., 2017).

D. Surface Plasmon Resonance-based biosensors

Liedberg et al. demonstrated SPR-based biosensors for biosensing for the first time in 1983 (Liedberg et al., 1983). Based on the concept of resonance changes caused by utilizing light waves to irradiate the substrate, SPR-based sensors operate. The changes in the resonance curves represent the valence electron's movement in response to direct stimulation (Liyanage et al., 2017). SPR is a technique that measures the refractive index of material adhered to the metal. The antibodies utilized in SPR immunosensing have been anchored onto a thin metallic layer, like gold. When immobilized antibodies and antigens interact, a change in the amount of light reflected from the back of the film is noticed, and the refractive index changes accordingly, allowing the concentration of the target analyte to be quantified (Englebienne et al., 2003; Iwasaki et al., 2001; Lee et al., 2003; Liedberg et al., 1995). Fig. 4 depicts a simplified SPR method in an optical biosensor (Cooper, 2002). SPR detection can overcome some of the disadvantages of traditional techniques, for instance, the prolonged analytic process, rising costs, and minimal bio-sensitivity (Han et al., 2016; Wu et al., 2017).

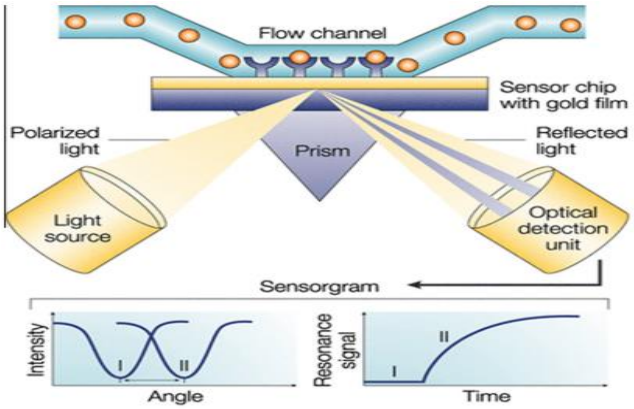

Fig. 4. The SPR technique in an optical biosensor (Cooper, 2002).

in their development, the sensitivity of cTn1 detection using SPR technique remains inadequate for clinical diagnosis, Despite significant advancements during biosensors progress, (Campu et al., 2022).

E. Surface-Enhanced Raman spectroscopy-based sensors

Compared to other optical techniques, enabling its precise identification, Raman spectroscopy offers information about the chemical structure of a molecule through its "fingerprinting" abilities, allowing its particular recognition (Le Ru & Etchegoin, 2008). Considering that the Raman effect is a weak phenomenon and has a low precision as well, mainly resulting from minimal scattering efficiencies, Surface-Enhanced Raman spectroscopy (SERS) can overcome this (Le Ru & Etchegoin, 2008; Vo-Dinh, 2008). Positioning the target molecules near a metallic surface increases the Raman signal, resulting in SERS (Campu et al., 2022). Raman and SERS function based on the concept of exciting the electrons from the metallic surface texture that has been exposed to a laser or another form of electromagnetic radiation. As biomolecules adsorb onto the surface of a metal with nanoscale roughness, the signal is enhanced. (Abdolrahim et al., 2015).

The SERS method allows for the specific detection and identification of the target biomarker due to its capability for fingerprinting (Campu et al., 2022). The SERS is an intriguing bio-sensing system due to its multiplexing capability and sensitive detection technique. The SERS foundation involves measuring

fluorescence based on the vibration of electrons, as a result, sharp and narrow bands are generated. These distinctive bands help to make SERS distinctive and specific (Cui et al., 2006). Furthermore, when analyzing highly complex biological liquids, significant background noise may be produced, causing the results to be altered (Campu et al., 2022). However, because metallic nanoparticles are used in this technique, there is still a challenge when imaging in vivo. These nanoparticles may become toxic over time (Vo-Dinh, 2008; Zhang et al., 2011).

3. Laser-induced fluorescence spectroscopy

3.1. A brief history of the fluorescence phenomenon

Any substance can emit light through a process known as luminescence, which results from electronically excited states. Depending on the nature of the excited state, luminescence is classified into two categories fluorescence and phosphorescence (Braslavsky, 2007; Herschel, 1845). Nicolas Monardes, a Spanish botanist and physician, was the first to recognize luminescence in 1565, he saw a blue shimmer in the liquid in a cup made of a particular kind of wood, Ligirium Nephiticiem. The renowned chemist Robert Boyle ran across the same issue in the following century (Bright, 1988). Sir David Brewster discovered a red emission from a green leaf solution in 1833. Although he had a different explanation for the phenomenon, nowadays it is known that the red emission is chlorophyll fluorescence. Herschel observed the first fluorescence emission spectra from quinine in 1845 (Ceredig, 2020). However, George Stokes was the one to suggest using fluorescence as an analytical technique in 1852. He also came up with the term "fluorescence". Goppelsrode carried out the first chemical analysis based on fluorescence in 1867, by creating a potent fluorescent Morin-aluminum complex, he created a technique for the trace detection of non-luminescent aluminum (Bright, 1988). Additionally, Adolf Baeyer used fluorescence in 1877 to show how the two rivers Rhine and Danube are connected; he dumped 10 kg of fluorescein, a chemical with powerful fluorescence, into the Rhine River, and three days later, the Danube River's green fluorescence was indicated, establishing the connection between the two rivers (Berlman, 1973).

3.2. Different de-excitation processes of excited molecules

The Jablonski diagram is typically used in many different ways to describe different processes that take place in excited states of molecules (Halappa et al.; Szudy, 1998). This diagram is named after Professor Alexander Jablonski, who is known as the father of fluorescence spectroscopy due to his many contributions, including descriptions of concentration depolarization and the trying to redefine the "anisotropy" to characterize the polarized emission originating from solutions (Halappa et al.; Szudy, 1998). Jablonski diagram shown in Fig. 5. In this case, S_0 stands for the singlet ground electronic state, S_1 is the first singlet excited electronic state, S_2 is known as the second singlet excited electronic state, and T_1 is the first triple excited electronic state (Albani, 2008).

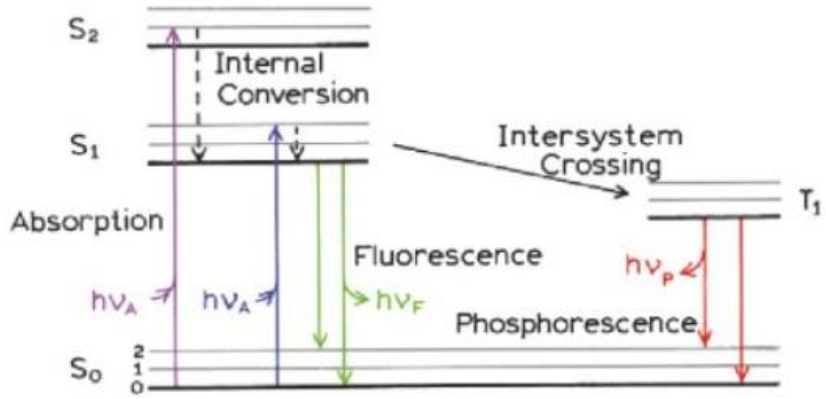

Fig. 5. The Jablonski diagram (Halappa et al.; Szudy, 1998).

Molecules (fluorophores) can exist at each of these electronic energy levels in a variety of vibrational energy levels, which are represented by 0, 1, 2, etc. after absorbing a photon (Joseph R Lakowicz, 2006). It's crucial to remember that absorption happens quickly (~ 10-15 s) compared to all other processes. Since most molecules exist in the lowest vibrational energy state of (S_0) at room temperature, now looking at potential de-excitation procedures that could follow (Joseph R. Lakowicz, 2006; Monge).

Once a molecule has been excited by photon absorption, it can return to its ground state by emitting fluorescence, internal transformation (i.e., returning to

the initial energy level without emission of fluorescence), or intermolecular charge transfer and conformational change, as well as intersystem crossing (perhaps followed by phosphorescence emission) (Makoui, 2007; Monge). The different processes of de-excitation are as follows.

3.2.1. Internal conversion

Vibrational relaxation allows the non-radiative decay of an excited molecule to a lower electronic state with identical spin multiplicity. Internal conversion is the name given to this process (Joseph R Lakowicz, 2006). For instance, when a molecule absorbs energy from the lower ground singlet state (S_0) to one of the vibrational levels of the excited singlet state S_n (n=1, 2, ...). The excited molecule positions itself at an unstable level, as a result, vibrating and losing some energy through internal conversion without emitting photons, and molecules quickly relax to the lowest vibrational level of S_1 (Albani, 2008; Anil & Anand, 2007; Hof et al., 2004). Internal conversion often takes lifetimes 10-12 s or less. Fluorescence lifetimes are frequently close to 10-8 s, therefore, internal conversion is usually finished before emission (Joseph R. Lakowicz, 2006).

3.2.2. Fluorescence and fluorescence emission properties

Fluorescence is established by, G.G. Stokes, in the 19th century (Valeur & Berberan-Santos, 2011). In the field of molecular spectroscopy, the word "fluorescence" is frequently used to describe the spin-permitted radiative transitions between the first excited singlet state and the ground state in which the excited orbital electron is paired with the second electron in the ground state orbital (of opposite spin). The fluorescence will occur if there is a transition between states of the same electron spin (Guilbault, 1967; Guilbault, 1973; Joseph R Lakowicz, 2006; Momose & Shida, 1998). In general, fluorescence is the process of absorbing light with a short wavelength and then emitting radiation with a longer wavelength. Because some molecules spontaneously de-excite from a higher energy level to a lower energy level, this radiation is known as fluorescence (Anil & Anand, 2007; Hof et al., 2004; Jaszczur & Pyrda, 2016; Walsh & Shakibaie, 2007). The fluorescence spectrum is positioned at longer wavelengths (lower energy) than the absorption spectrum, due to energy loss in the excited state caused by vibrational relaxation (Monge). The energy difference

between the ground state and the excited state is equal to the energy of the electronic transition. Thus, the energy transition, E (J.mol-1) is as follows:

$E = h\nu \qquad E = hc/\lambda \qquad (1)$

the wavelength is λ measured by (nm), the light frequency (ν) is by s−1 or Hertz, Planck constant (h) equal 6.63×10−34 J·s, and c is the velocity of light. As a result, each transition takes place at a certain single wavelength and with a particular energy. However, spectra are observed rather than individual lines donating to the de-excitation and absorption energy due to the vibrational and rotational energy states (Albani, 2008). Typically, the UV or visible range of the spectrum is where the radiation is emitted, with a fluorescence lifetime of approximately 10 ns (Bernath, 2020). The molecule can return to its ground state either through intersystem crossing or fluorescence depending on the number of vibrational levels existing, the relative power of the optical transitions and relaxation transitions, and the energy gaps. The fluorescence emission has some characteristics as follows:

A. The stokes shift

The emitted photon energy is often smaller than the energy molecule absorbs according to the Jablonski diagram shown in Fig. 6. Fluorescence occurs at lower energy and longer wavelengths. Sir G. G. Stokes was the first to notice this behavior in 1852, so it has been known as the Stokes shift (Stoke, 1852; Stokes, 1852), as illustrated in Fig.(Monge).

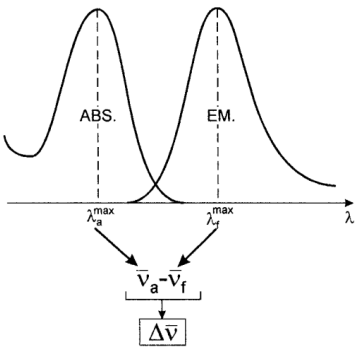

Fig. 6. Definition of the Stokes shift (Stoke, 1852; Stokes, 1852).

Stokes shift is defined as the gap between two bands, the maximum of the first absorption band, and the maximum of the fluorescence spectrum (expressed in wavenumbers) $\Delta\bar{\nu} = \bar{\nu}_a - \bar{\nu}_f$ (Albani, 2008). Equation (2) shows the absorption energy

$$E_a = h/\lambda_a \quad (2)$$

The emission of energy is $E_{em} = hc/\lambda_{em}$ (3)

Since $E_{em} < E_a$ (4)

We have $\lambda_{em} > \lambda_a$ (5)

The wavelength of the absorption peak is (λn_a) and the wavelength of the emission spectra peak is (λ_{em}). As a result, the maximum of the emission spectrum is pushed to longer wavelengths than the maximum of the absorption spectrum (Albani, 2008).

B. Emission spectra are generally not dependent on the wavelength of excitation.

Fluorescence has another feature, which is the same emission spectrum is generally seen regardless of the excitation wavelength; this phenomenon is known as Kasha's rule. (Kasha, 1950). The excess energy is immediately dissipated upon stimulation into higher electronic and vibrational states, the molecule remains in the lower vibrational state (S_1). The relaxation process takes around (10–12 s) and is thought to be due to a large overlap between many equal energy levels. Emission spectra are frequently independent of the excitation wavelength due to this quick relaxation. There are exceptions, two ionization states fluorophores, each state has absorption spectra and emission spectra. Furthermore, some compounds can emit at the S_2 level, but this is uncommon in biological molecules (Birks, 1970).

C. Mirror–image rule

When the chance of the S_1 - S_0 transition is the same as the likelihood of the S_0 - S_1 transition, the emission spectrum of a fluorophore is the mirror image of its absorption spectrum. However, if fluorophore exciting results in an (S_0 - S_n)

transition and internal relaxation will take place, causing the molecules to enter the initial excited singlet state before emission, this will cause a distinct emission transition from the absorption transition (Albani, 2008). The fluorescence spectrum frequently resembles the first absorption band because, in general, the differences between the vibrational levels in the ground and excited states are similar (the "mirror image" rule) (Monge).

D. The lifetime of fluorescence

Following exciting processes, molecules remain in the excited energy level and then de-excited to the ground energy level. The lifetime of the excited energy level is a measure of how long molecules typically remain in the excited energy level. The fluorescence lifetime is thought to be at this time. The time spans between picoseconds (10-12 s) to nanoseconds (10-9 s) (Albani, 2008).

3.2.3. Intersystem Crossing

The intersystem crossing is a non-radiative transition between two isoenergetic vibrational levels associated with electronic states of various multiplicities (Monge). For example, when an electron in an excited singlet state reverses its spin, it transits to the triplet state vibrational level (from S_1 to T_1) (Bernath, 2020). The rate constants for triplet emission are several orders of magnitude smaller than those for fluorescence because the transition from T_1 to S_0 is prohibited (Joseph R. Lakowicz, 2006). Other de-excitation processes from S_1 may compete with intersystem crossing, which is quick enough (10-7-10-9 s) (Joseph R. Lakowicz, 2006; Monge).

3.2.4. Phosphorescence

The term "phosphorescence" refers to the transition from a higher energy level to a lower level of a different multiplicity (Joseph R. Lakowicz, 2006). Where electron spin is first exchanged by intersystem crossing from S_1 to T_1 directly below S_1 (Anil & Anand, 2007; Joseph R. Lakowicz, 2006). Spine orientation is necessary for phosphorescence, it happens between states with different spins (Anil & Anand, 2007; Hof et al., 2004). These radiative transitions are characterized by relatively long lifetimes between milliseconds and minutes since they are spin-prohibited (Joseph R. Lakowicz, 2006). The vibrational level

of the T_1 is lower than that of the S_1, and the spectrum of phosphorescence is seen at longer wavelengths than the spectrum of fluorescence (Joseph R. Lakowicz, 2006; Monge).

3.2.5. Delayed fluorescence

There are different types of delayed fluorescence as follows:

A. Delayed fluorescence thermally activated

Reverse intersystem crossing from energy state T_1 to the energy state S_1 can happen when the difference in energy between the two states is minimal and the lifetime of T_1 is long enough. The emission has the same spectral distribution of the emission is similar to conventional fluorescence with a significantly longer decay time because the molecules remain in the T_1 before emitting from S_1 (Monge). It was first noticed with eosin; it is known as E-type delayed fluorescence (Monge).

B. Triplet–triplet annihilation

In concentrated solutions, the energy released from molecules collision in the triple electronic level may be sufficient for one of them to transition back to the S_1 state. As a result, a delayed fluorescence emission results from the triplet-triplet annihilation, which is also known as P-type delayed fluorescence because it was initially noticed by pyrene (Monge).

C. Triplet–triplet transitions

Triplet-triplet transitions are spin-allowed, consequently, when the molecule is excited and stays in T_1, it can absorb additional photons with specific wavelengths. These transitions can be seen if there are enough molecules in the triplet state, which can be achieved by illumination with an effective laser pulse (Monge).

3.2.6. Another de-excitation processes

De-excitation may be displaced by interactions between molecules in the excited state such as electron transfer, proton transfer, energy transfer, and the production of an excimer or exciplex (Albani, 2008; Monge).

3.3. Fluorophores

The fluorophores are tiny molecules that are present in a variety of cells, making them useful natural markers for studying living cell's structure, metabolism, and dynamics (Albani, 2008; Joseph R. Lakowicz, 2006). They are the main component of fluorescence spectroscopy; the fluorophores of molecules are the elements that give them fluorescence properties. Tyrosine, Tryptophan, phenylalanine, fluorescein, and other molecules with aromatic rings are the most common fluorophores (Bose et al., 2018). These aromatic amino acids are the source of intrinsic protein fluorescence (Herschel, 1845; Hoskin, 1994; Udenfriend, 1995). Proteins absorbance and fluorescence in the UV due to these amino acids (Albani, 2008; Joseph R. Lakowicz, 2006). As a result, fluorescence spectroscopy is an extensively used technique in the investigation of proteins (Coates, 2000). Fluorophores are two main kinds. The first is intrinsic (natural) fluorophores as aromatic amino acids and cofactors. The second type is extrinsic fluorophores that can be covalently or non-covalently bonded to macromolecules (sample) but do not exhibit the intended spectrum characteristics (Albani, 2008; Joseph R. Lakowicz, 2006). The fluorophore can be identified by its emission wavelength, emission intensity, position, and emission lifetime. Each fluorophore has unique observable and fluorescence characteristics. These characteristics are built into the fluorophore and change depending on the surroundings. The fluorescence spectrum is a graph showing the intensity of fluorescence as a function of wavelength (Albani, 2008).

3.4. Fluorescence spectroscopy applications

There are numerous fluorescence spectroscopy applications such as Clinical /Cytochemistry, Life science /Lipid studies, Environmental /Enzyme analysis, Oceanography /Occupational chemical hazards, Pharmacology /Public health, Abused drugs /Agro-chemical analysis, Toxicology /Trace-element analysis, Radio-dosimetry /Rare earth analysis and Antigen, antibody /Amino acid assay (Singh, 2016). Additionally, the application of fluorescence spectroscopy in biological sciences has increased significantly over the last 20 years and fluorescence has become more widely used. As well as it is commonly used in biochemistry and biophysics as a research technique (Joseph R. Lakowicz, 2006). It has been effectively used to identify proteins, enzymes, DNA, RNA,

and other biomolecules (Bright, 1988). Fluorescence spectroscopy is recognized as one of the most effective methods for investigating the formation and function of biological molecules, especially proteins (Eftink, 2002). Weber (Teale, 1957; Weber, 1952), Teale (Teale, 1960; Teale, 1957), Konev (Konev et al., 1967), Burstein (Burstein et al., 1973), Brand (Beechem & Brand, 1985) and their numerous protégés and colleagues (Biomolecules, 1989; Cundall, 2013; Demchenko, 1981; Eftink, 1991; Joseph R Lakowicz, 2006; Longworth, 1971) demonstrated that proteins can emit luminescence when stimulated by ultraviolet radiation. Furthermore, These studies have revealed that protein fluorescence could provide various information including, intramolecular distances, the degree of rotational motion freedom, and the exposure of amino acid side chains to quenchers (Eftink, 2000). Additionally, protein structure is frequently detectable in the emission spectra of proteins (Lakowicz et al., 1986). Several fluorescence spectroscopy techniques have been utilized for disease detection, biological, and biomedical research, in recent years, including LIF (Al-Salhi et al., 2011; Benmansour et al., 2011; Ducháč et al., 2011; Silveira et al., 2008).

4. Brief history and basic concept of laser-induced fluorescence spectroscopy

The LIF has a long and illustrious history, the induced fluorescence phenomenon was first observed and described by R. W. Wood in 1905 several decades before the advent of the laser (Daily & science, 1997). Lannuzzi. (Iannuzzi & Polacco, 1965) first published a paper in 1965 on the polarization of LIF in anthracene. After that, in 1970, LIF was employed to measure the spectra of numerous compounds, and elements (Johnson et al., 1970). Hickman and Moore employed LIF in algae and rhodamine (HICKMAN, 1970). Since 1997, LIF has been used in the field of biomedicine (Kohl et al., 1993). The LIF is defined as the spontaneous emission of atoms or molecules that have been excited using a laser source (Daily & science, 1997). The following is a summary of the advantages and applications of LIF.

4.1. Laser-induced fluorescence advantages and applications

Selectivity; in addition to the selectivity of laser absorption, a further selection is obtained by fluorescence observation (Giorgio Dilecce et al., 2015). Fluorescence spectroscopy is a very sensitive method because the photons that are released are detected against a negligible (dark) background. Fluorescence has a sensitivity that is 100–1000 times greater than absorption techniques, allowing for the measurement of concentrations in parts per billion levels (Sikorska et al., 2012). Therefore, fluorescence detection sensitivity and most biochemical measurements no longer require the cost and difficulty of using radioactive materials (Joseph R. Lakowicz, 2006). It is a desirable technique for isolating fluorescent components from complicated mixtures (Bright, 1988). LIF is therefore employed as a diagnostic and analysis tool (Ramanujam, 2000).

The fluorescence technique requires very little material, only nanomoles of the analyte are required in a typical fluorescence measurement (Eftink, 2000). Additionally, because of the link between the concentration of the sample and the fluorescence intensity, the sample concentration could be evaluated (Gabbarini et al., 2019). Time resolution, the duration of the laser pulse affects time resolution, and the femtosecond time scale can be used for shorter pulse durations (G Dilecce et al., 2015). The detection limit of LIF, as a type of high-efficiency analytical technology, can approach 10-13 mol/l (Liu et al., 2013).

The several advantages of LIF spectroscopy have made it a powerful technique with a wide range of uses. Applications in a wide range of fields have been reported, including the detection of tumors, oils, and drug analysis, and monitoring air and water pollutants (Monge; Watson et al., 2011). The LIF is one of the most sensitive methods for qualitative and quantitative analysis (Patil et al., 2012). Additionally, LIF may be a useful strategy for creating a tool that can analyze and categorize infections, as different viruses produce different spectra as fingerprints, allowing for their classification, due to differences in their molecular structure (Gabbarini et al., 2019). Furthermore, fluorescence detection-based analytical methods are widely used. A spectrofluorometer that operates at the proper excitation and observation wavelengths can be used for direct fluorometric detection of fluorescent analytes. This is true of proteins

(such as those found in blood serum), drugs, aromatic hydrocarbons, chlorophylls, and so on (Monge). Optical fibers are used in LIF to deliver, collect radiation, and couple photons with spectrometers (Drakaki et al., 2014). The early 2010s saw a considerable improvement in LIF technology, due to several advancements in the field of spectral instrumentation. This was made possible by the presence of a variety of reliable, low-cost, palm-sized spectrometers with high spectral resolution and sensitivity (Marques da Silva & Borissovitch Utkin, 2018). Surprisingly, spectrometers are produced for a wide variety of applications (Hu et al., 2005). The majority of the lasers used in LIF measurements are pulsed, with pulse durations of a few nanoseconds or less. Their main advantage over continuous wave (CW) lasers is that they have a higher power (Bras, 1990). Hundreds of different laser systems have been developed to generate pulses ranging from milliseconds to femtoseconds (Peshko, 2012).

The femtosecond laser is one of the most significant advances in laser technology (Zhang et al., 2019), developed during the 1980s. Femtosecond lasers are a clear technological accomplishment with great possibilities for a wide range of applications, and they have made significant advances in the study of light-matter interaction (Tan et al., 2016). A previous study revealed using femtosecond LIF spectroscopy in detection of HSA which is a biomarker in diagnosis liver and kidney diseases (Mohamed et al., 2022). In this study, the standard calibration curve was conducted while the wavelength of excitation changed and HSA concentrations were systematically changed, and the spectra data of LIF were obtained. The excitation wavelength equal 350 nm revealed the highest fluorescence emission and the centered wavelength of the fluorescence peak is at 500 nm throughout a broad range of excitation wavelengths. A comparison of the measurement of specific HSA concentrations utilizing the LIF technique and traditional methods was conducted. A previous study revealed the use of femtosecond laser-induced fluorescence for rapid monitoring of cardiac troponin 1 as a cardiovascular disease biomarker (Mahmoud et al., 2024). In this study, an excitation wavelength, ranging from 350 nm to 400 nm, was used to excite cTn1 with constant concentration (8 ng/ml), and the spectra data of LIF were subsequently obtained. The obtained results indicated that at 350 nm

excitation wavelength, the fluorescence peak intensity reached its highest, and the central wavelength of the fluorescence peak was at 494 nm. Using an excitation wavelength equal to 350 nm, different cTn1 concentrations were examined and LIF spectra data were collected. The obtained results showed that the fluorescence peak intensity is concentration-independent and linearly increases as cTn1 concentration increases. These studies (Mahmoud et al., 2024; Mohamed et al., 2022) demonstrate that using femtosecond LIF may be a distinctive, selective, accurate, effective, and direct method for HSA and cTn1 monitoring.

5. Conclusion

In this article, we have explained a brief overview of the importance of biomarkers focused on proteins, and their function, and introduced some protein examples. HSA is used as kidney and liver biomarkers, and cTn1 is a cardiovascular disease biomarker. A summary of the improvements made in the HSA and cTn1 using different analytical techniques was introduced. The benefits and disadvantages of certain biomarkers detection methods were discussed, demonstrating the development of high-throughput detection systems. We introduced an overview of additional challenges, using LIF spectroscopy as one of the spectroscopic techniques based on fluorescence. Furthermore, the physical meaning of the fluorescence phenomenon and different de-excitation processes of excited molecules were discussed focusing on the application of fluorescence spectroscopy. An overview of the history and basic concept of laser-induced fluorescence spectroscopy, advantages and disadvantages of this technique that has a wide of applications introduced. Furthermore, LIF can simultaneously as a rapid and simple method to detect HSA and cTn1 in blood without requiring sample preparation like centrifuging or adding chemicals. Using a femtosecond laser system can offer the benefit of adjusting parameters like power, wavelength, and beam radius to attain optimal outcomes. These advantages are not found in other laser systems.

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