Application of fluorescence-based analysis methods

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Abstract. Fluorescence has been discovered over decades and enormous fluorescence technology appear and been applied to different field. This article mainly focuses on the fluorescence technologies with good function or potential that has relationship with health. The field of the health is extremely broad, so we just summarized it into two parts, one is the cause of unhealthy, one is the restore health. Apart from those physical injuries, food safety is a major risk of un-wellbeing, so to effectively improve the accuracy and efficiency of the detection technology of contaminants is very important, where metal-organic frameworks (MOFs) can improve this. Fluorescent imaging and other fluorescent biosensors have great abilities and potential in clinical technology and biomedical field to help to restore healthy. While not just these fluorescence technologies themselves need to discuss, fluorophore is also an essential part. As most fluorescence technologies need to use it. As a result, this research shows the application of fluorescence-based analysis methods in different fields, including food safety and biomedicine.

Keywords: fluorescence, biosensor, biomedicine, application

1. Introduction

Fluorescence was not that popular as it was first been observed. Only 1970 documents were recorded during 19 centuries in Google scholar. Sir Herschel is the first scientist record the observation of the fluorescence and finds that the quinine solution which is colourless can emit the blue light in 1845 [1]. But the term of "fluorescence" is noted by George Stoke in 1852. He explained more detail about Herschel's work and been named for Stoke shift. It is another important term in the fluorescence field. Scientists start to put the tension on the fluorescence as its high sensitivity and quantity. The companies Carl Zeiss and Carl Reichert realized the first fluorescence microscope. And Ellinger and Hirt design the "intravital microscope "which is the origin of the modern fluorescence microscope. In early 1940s, fluorescent antibody labelling was discovered. In 1990s one of the most famous fluorescents has been found in jellyfish Aequorea Victoria, green fluorescent protein (GFP). After that, a diverse of different fields benefited from the development of the fluorescence. For example, the cell biology, food safety detection, medical and biochemical research. These are all important to modern human life and the future development of the scientific fields.

Fluorescent biosensors based on the change in fluorescence can be used to provide reports of interactions between protein and protein, changes in conformation, enzyme activity and post-translational modifications [1]. Fluorescence-based analysis method preserves the sample and the molecules of interest within it, which is therefore suitable for non-destructive imaging. Scientists can track molecules moving in complex solutions or environments in a real-time manner with the support of the high degree of temporal and spatial resolution provided by imaging of fluorescent signals.

Consequently, fluorescent biosensors can be used to monitor dynamic molecular events and visualize dynamic processes. And fluorescent biosensor is the beneficial tool for the detection of biomolecules. Fluorescent biosensors can detect different analytes by varying parameters such as the intensity of fluorescence. Based on these properties and advantages, fluorescent biosensors have been widely used in different fields, including biomedicine and food safety. The most common application is to identify different substances by comparing spectrum and detect the amount of certain substance contained in solution. Moreover, structure and conformation of macromolecules, DNA sequences detecting and energy transfer in macromolecules are also involved in florescent technologies.

Despite the progress achieve in existing research, there are other regulation effects of fluorescence technology needing to be explored. For metal-organic frameworks (MOFs), various fluorescent probes can be integrated into MOFs, realizing detection of different targets. For fluorescence lifetime imaging microscopy (FLIM), as new optical instruments and analysis methods, the application of FLIM has broadened, and there is some FLIM-assisted clinical cancer diagnosis and tumor boundary determination cases. For luciferases, enzyme activation can be applied to design various fluorescent biosensors with high sensitivity and selectivity. In this research, different types of applications of fluorescence will be mentioned and introduced detailly, including fluorescent biosensors, drug discovery and Fluorescence imaging.

2. Applications of fluorescence-based analysis methods

2.1. Fluorescent biosensors

Fluorescent biosensors can detect the markers required for research in complex environments, which has been widely used in a diverse of various fields. Y-box binding protein 1 (YB1) is a known marker of metastasis. In the nucleus, YB1 interacts with human endonuclease III (hNTH1) and regulates its activity [2]. A förster resonance energy transfer (FRET)-based fluorescent biosensor capable of adapting high-throughput screening (HTS) technology was designed, as shown in Figure 1.

This kind of fluorescent biosensor performs FRET-based HTS assays. It consists of a fused polypeptide chain encoding both a fluorescent tag and a target protein (Figure 1). In more detail, the fusion protein polypeptide chain consists of super yellow fluorescent protein 2 (SYFP2)-C-terminally truncated form of Y-box binding protein 1 (YB1ΔC)-hNTH1-mTurquoise2 (mTQ2), where SYFP2 is positioned at the amino terminus of the construct [3]. Based on experimental data from this fluorescent biosensor used in the HTS search for chemical inhibitors of the hNTH1-YB1ΔC1 complex, the minimal amount of biosensor required to measure FRET reliably is 0.2 μM and the biosensor for the HTS assay is compatible. A dose-response curve with THF-oligo (providing hNTH1-YB1ΔC interaction) showed that the difference in FRET values remained at ~20 % in the presence and absence of THF-oligo. The large change in the FRET signal makes this assay suitable for HTS. In summary, this kind of FRET-based fluorescent biosensor is simple, fast, and robust for HTS, which demonstrates the FRET-based biosensor is a promising technology for drug discovery applications.

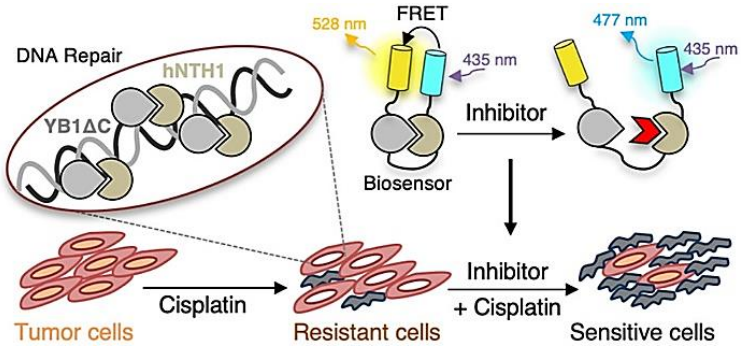

Figure 1 Scheme of the developed FRET-based fluorescent biosensor [3].

Fluorescent biosensors can detect the markers required for research in complex environments, which has been widely used in a diverse of various fields [1]. RNA undergoes several modifications after synthesis in the cell. Among these, N6-methyladenosine is the most common modification, and some cancers are associated with abnormalities in N6-methyladenosine modification in which demethylases act as oncogenes [4]. The demethylases AlkB homolog 5 (ALKBH5) is one of the demethylases involved in N6-methyladenosine modification. Therefore, a fluorescent biosensor was used to effectively quantify the activity of the RNA demethylase ALKBH5 in order to find an effective inhibitor of the demethylase. This fluorescent biosensor is based on a *E. coli* toxin MazF/nano-graphene oxide (NGO) hybrid system for enzyme activity detection of ALKBH5, where MazF is the initial detector for detecting the activity of the target enzyme and NGO acts as an effective quencher and transistor [5].

The remaining single-stranded oligonucleotides adsorb to the NGO surface, resulting in a fluorescent dye burst and a weak fluorescent signal. The inactive ALKBH5 is unable to remove the methyl group, failing MazF-related cleavage, and a strong fluorescent signal is detected when the NGO is added to the RNA hybridisation with its complementary DNA probe, as shown in Figure 2. The Z' factor of this fluorescent biosensor was calculated to be 0.84, showing a possibility for high throughput screening assays. In summary, this fluorescent biosensor not only enables effective analysis of demethylase activity and rapid detection of its inhibitors but also plays a role in drug discovery.

Luciferase is enzymes that produce light when they oxidize their substrate. It is used as a clear indicator in assays because it produces coloured light which can be observed. Besides, different luciferases produce light in different colours so that scientists can increase accuracy of experiment data based on internal control [6]. And this idea is used in dual-luciferase report assay. Dual-luciferase reporter assay is high sensitivity, wide dynamic range and flexible application. Firefly luciferase and renilla luciferase are involved in this technology. The renilla luciferase acts as internal control which is mentioned before and emits blue light of 465 nm wavelength [7]. The internal control is possible to minimize inherent variability that can undermine experimental accuracy [8]. Plasmid is a kind of nucleic acid molecule which can replicate independently and be stably inherited. After transfection and cell cracking, the intensity of florescence can be detected by florescent detecting devices [9]. The chemical property of fire luciferase makes it important for detecting the presence or concentration of ATP. There is a linear relationship between the number of microorganisms and the amount of ATP. The luciferase assay has been shown to have a sensitive range about the detection of approximately 10^{-14} M ATP [10]. This leads to another technology in food, ATP bioluminescence technology.

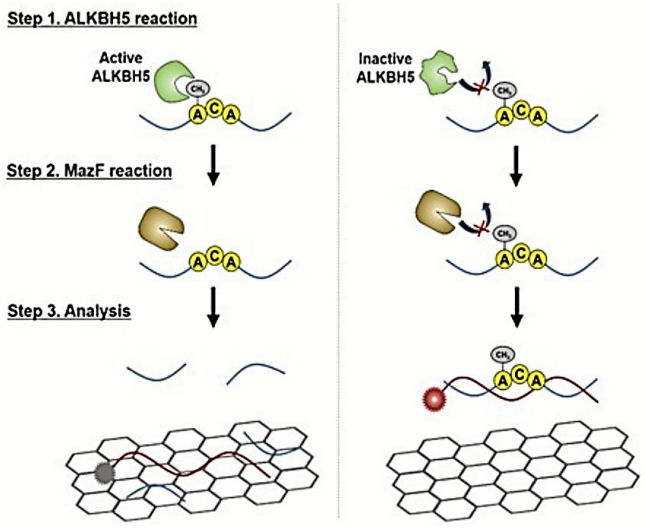

Figure 2 Scheme of the used fluorescent biosensor [5].

ATP bioluminescence technology requires no culture process, is easy to operate, has high sensitivity, and can obtain results in a few minutes [11]. Usually, the fluorescent labelling of cell,

bacteria, virus, gene helps scientists to build disease models influenced by certain antigens. The advantage is that monitoring in real time to cellular activity in the same organism instead of conventional method. The presence of technology causes the great observation of cancer cells in invading and metastasizing. Based on their own features, they are different from both mechanisms and applications. The near absence of background signals or auto fluorescence increases the sensitivity compared with florescence imaging, but BLI can only produce macroscopic imaging due to restriction of fluorescent brightness and less spatiotemporal resolution [12]. By integrating Fluc gene into cell chromosomal DNA to express luciferases, when substrates are injected into body, the luciferases start to work and emit the number of photons which human eyes cannot observe. This makes us need ultra-sensitive charge-coupled device (CCD) cameras to capture the photons emitted and demonstrate the real time graph with the specially designed camera obscura and imaging software [13]. The depth of detection reaches three to four centimetres.

However, the BLI still has disadvantages. For example, the cost of BLI becomes higher than florescence imaging by reason of substrate needed. All luciferases need the presence of plenty of oxygen to emit photons, so catalysing rate in acutely hypoxic tissues or dead organisms may be affected. The new luciferase can lead us to go deep into BLI through its high affinity to substrates and producing more photons for observe in a certain unit time [14]. Furthermore, visualization of cancer cell signalling is realized by this method also. Out of oncology, BLI shines in other areas as well.

2.2. Fluorescence imaging

Fluorescence lifetime imaging microscopy (FLIM) is a widely used imaging technology in life science as it has high specificity and high sensitivity. It is not only can localize the fluorophore, it also can detect the surrounding environment of the fluorophore in single molecular level and the molecular conformation done to their fluorescence lifetime. There are plenty of problems need to solve as the rapid development and innovation of the scientific technologies and discoveries. But it can be generalized as three main issues: the imaging speed, accuracy of fluorescence lifetime measurements and resolution of the image.

The theory applied by FLIM is very simple, it just based on the principle of fluorescence. Fluorophore will absorb the photon emitted by laser and been excited from ground state to the higher single state. Then it will go back to the ground state after the radiative or non- radiative process. Then photon will be released by fluorophore during the radiative process. This is a detectable phenomenon which can be record by the specific machine. And the average time used before the arrival of photon is the fluorescence lifetime. The basic equipment to measure it are single photon detector and photon time-gated technology. Because of the size of the photon is in picometer, the amplification is needed. Photons will impact a photovoltaic to generate electrical current to increase the size. This photon signal will be detected by the time gated machine and transfer into digital signal for further lifetime analysis [15].

And on this basis, FLIM measurement can be separated into two types [16], as shown in Figure 3. One is frequency domain, and the other is time domain. FD-FLIM is based on the modulation technology for generating the oscillation wave. The pulsed laser will emit an oscillating wave and the angle of the frequency of this wave is reciprocal to the lifetime of the sample that measured. At each point in time the sample has been excited it will give a decay profile. After this it will result in another oscillating wave which called convolution. The emission wave will be delay in time and demodulated as photon takes time to re-emitted.

To obtain the parameters of the relevant of the decay profiles, apparent lifetime is needed, and it can relate to the phase shift lifetime. Also, the modulation lifetime is needed which related to the demodulation. However, the approach used to measure the frequency domain is usually streak camera, where the open and close of the camera need to be at the same frequency as the excitation wave. And then integrate over many different peaks, the intensity should be specific in different phase position. And the intensity as function of the phase step shown in here.

TD-FLIM is a simple photon counting approach which include streak camera, time gate techniques and TCSPC. It is directly measured the histogram in time of the photon distribution after you excite the sample. TCSPC-FLIM is one of the prevalent TD-FLIM. It was first born in later 1980s after the combination of the ultrafast pulsed laser and scanning microscope. And because of the enormous amount of photons requirement to do accurate measurement, its imaging speed become one of the shortages. However, as TCSPC has been record approximately 30 years, scientists have made a lot of improvements to this technology. For example, wide-field imaging, SPAD, can all accelerate the speed of construction of image [15]. For now, TCSPC-FLIM is a very mature and robust methodology for imaging in clinical, medical and biological area, e.g., cancer and tumor detection field.

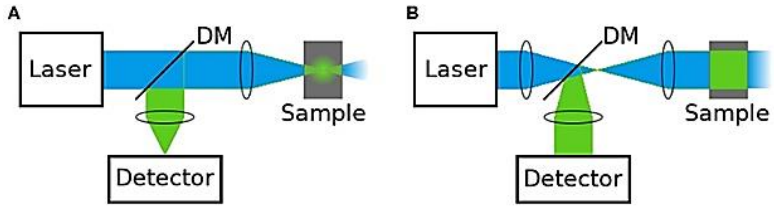

Figure 3 Schematic diagram of two types of FLIM [16].

FLIM can be used in various field, but most in medical and clinical area. As health and longevity is the most inescapable and popular topic to do research. With the improvement of the technology, human lifetime increased significantly. In 2019, the global average age at the death was 72.6 years. Surgery and chemotherapy are the basic treatment of the cancer. For surgery, how to ensure that the removed tissue is a tumor rather than healthy tissue is a great attention to clinical research. As tumor can grow in the any organs in the body, it is important to preserve as much healthy tissue around the tumor as possible for the patient's postoperative recovery of the organ function. Tissue classifiers based on FLIM parameters have shown potential to differentiate healthy from cancer tissue in Head and Neck (H&N) cancer patients [17]. While some type of cancer not just rely on the surgery and chemotherapy but also rely on the other treatments, such as the hormone therapy of the breast cancer. Because there are 80% of patient suffer from the ER-positive tumors, the hormone therapy is the first option. But in the long-term, the side effect of the treatment will cause the relapse of the cancer and the drug resistance. The hormone therapy is no longer useful and so did those basic treatment. They need more specific and deep investigation of the treatment e.g., targeted molecular therapy. But the traditional method used to screen the target molecule in the therapy [18]. However, the combination of FLIM and FRET can solve the inefficiency and accuracy of the targeted molecular therapy done to the property of the FRET and FLIM. It can detect the direct interaction of the ER with the different chemical compounds as its resolution is in single molecular level and FRET is not easily influenced by the photobleaching. FLIM can observe the potential therapeutic target of the breast cancer. In future, it has the potential to screen epigenetic targets simultaneously to achieve high throughput and to screen a range of another nuclear receptor-related target.

2.3. Food safety analysis

With the improvement of people's quality of life and continuous population growth, food security issue has become seriously important for human society. At the rest excessive use of food additives and pesticide residues are the key factors restricting food safety. Traditional techniques like included atomic absorption spectrometry, atomic fluorescence spectrometry, inductively coupled plasma-mass spectrometry gas chromatography-mass spectrometry (GC-MS) and ion-mobility spectrometry (IMS) have been widely used to food safety analysis [19]. However, the complicated technology equipment restricted the widespread applications. As a result, MOFs-based fluorescent biosensors are developed and used for food safety analysis [20-22].

Water is one of the most important ingredients in food, and water can react with compound in food system. In addition, it's a main factor that accelerate the soilage of food. Hence, it's necessary to monitor the quality of water in the food source. The MOFs-based sensor can be used to sense humidity. Using this method, a luminescence quenching effect is obtained by the encapsulation of water

molecules at different time intervals when the relative humidity reaches 33%. The color of MOFs is changed, proving that is a highly valued humidity sensor. As well as there is great potential of other application, a test strip is prepared with Ru@MIL-NH_2 applied into detection of trace water in white spirits. To sum up, MOFs has huge potential in food, thus effectively curb food spoilage, to in ensure the food safety [23-25].

Protein is one of the seven essential nutrients for the human body, amino acids are the basic building blocks of protein as well as one of the most crucial ingredients in food. Also, MOFs-based fluorescent biosensor can be used in detecting amino acids, as shown in Figure 4. A dual-emission Eu-MOFs fluorescent biosensor shows good sensing performance for Asp and Glu [26]. The results exhibit that the detection limit for Asp and Glu are 21 μM and 22.4 μM, respectively.

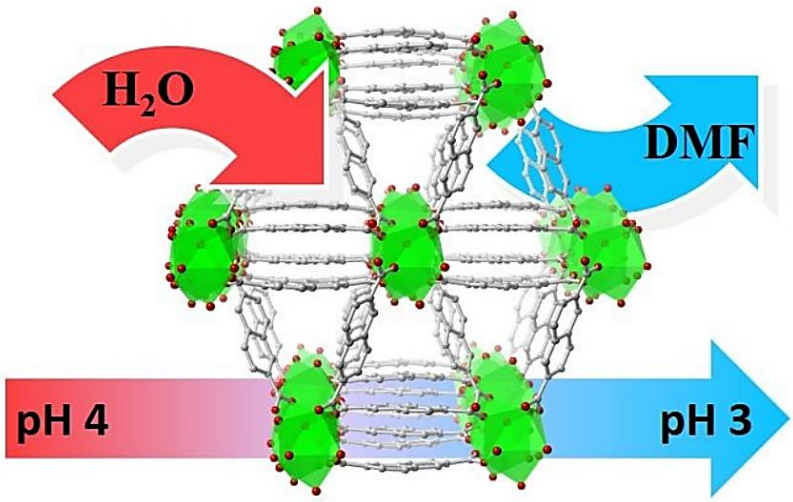

Figure 4 Scheme of the used pH-modulated luminescence [26].

3. Conclusions

The aim of this research is to summarize the application of fluorescence technology in the variety of fields and highlighted the fields of food detection, drug discovery and fluorescent imaging. Fluorescence technology had outstanding performance in multiple areas benefited from high sensitivity of fluorescence to change in molecular structure, chemistry and local environment. However, fluorescence technology faces some challenges. For example, in food detection area, it needs complex experimental steps to purify sample and enrich the targeted analytes to achieve a reliable detection. Simultaneous integration of preconcentration and detection will be a promising trend in food safety analysis in the future.

Authors' Contributions

These authors contributed equally.

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