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Principles and applications of fluorescent probe imaging technology

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Abstract. Fluorescent probe technology was discovered in the 19th century and imaging techniques were applied to microscopy around 1670. Fluorescence microscopic imaging is a classical method for observing the structure of organisms in the life sciences. In this regard, single-molecule fluorescence imaging uses fluorescent probes to label, detect, and analyze individual molecules, helping scientists to clearly observe the activities of individual molecules without disrupting the normal physiological state of the organism. In this paper, the principles and applications of fluorescent probe imaging techniques are described and analyzed. The common detection methods of fluorescent probes are spectrophotometry, electrochemistry, atomic absorption spectroscopy, high performance liquid chromatography, and fluorescence spectroscopy. Rapid detection results can be obtained depending on the specific method.

Keywords: fluorescent probe technique, imaging, microscopy, fluorescent protein, biology field.

1. Introduction

Fluorescence microimaging is a classical method for observing the structure of organisms in the life sciences. In this regard, single-molecule fluorescence imaging uses fluorescent probes to label, detect, and analyze individual molecules, helping scientists to clearly observe the activities of individual molecules without disrupting the normal physiological state of the organism.

Fluorescent probes are used in a wide variety of assays and labeling applications, such as determining metal ions, pesticide residues, biomolecule content, tracking biomolecules, labeling macromolecules, and cellular and subcellular structures. With the rapid development of life science and technology and the advent of today's era, emerging biochemical sensors represented by organic small molecule fluorescent probes are increasingly used in environmental monitoring, life science, and pharmaceutical research.

Therefore, this study uses the literature reading method as well as the comparative study method to conduct preliminary research on fluorescent probes. This paper is divided into four parts. The first part briefly introduces the basic concepts of probes and fluorescent probes; the second part describes the principles of fluorescent probes from three aspects, such as the luminescence mechanism, detection molecular properties, and signal processing analysis; the third part explores the application scope of fluorescent probes in terms of spectroscopy, imaging, and other applications; the last part concludes with the research implications and outlook.

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In this paper, the importance of fluorescent probes in biology is initially investigated from both the principles and applications of fluorescent probes. Nowadays, the accurate detection of pH is very important in the scientific field, so many detection methods have emerged, such as acid-base titration detection and potentiometric titration detection. However, most of these methods have the disadvantages of low sensitivity, high cost of use, uncomplicated operation, and susceptibility to interference by other ions. Compared with traditional methods, fluorescent probes can have a very wide range of application, user-friendly operation, and more accurate results. Most importantly, biofluorescent probes can enter individual cells for precise detection, which is of great importance for the development of modern biotechnology. Therefore the fluorescent probe for pH detection is an excellent choice [1].

2. Background information

2.1. Probe

The probes are composed of many individual molecules and biomolecules, each composed of many different molecules. The nature of the molecules is related to the fluorescence effect. Molecules that are conducted with fluorescent signals during molecular recognition are called fluorescent sensors, i.e., fluorescent probes. Because fluorescent probes are sensitive, easy to operate, and have good selectivity, they are widely used in the detection of molecules and ions, detection of bioactive substances and cell imaging, near-infrared fluorescence and time-resolved tests, etc. In order to determine whether fluorescence exists in a specific region of a cell, fluorescent probes are needed to determine the nature of biomolecules [2].

Three types of fluorescent probes are commonly used: first, disulfide bond (R) probes and molybdenum disulfide (MgO) probes; second, covalent bond (M (Cu) bond, R) probes and olefin fluorescent probes; and third, isotope labeling of dipoles (Pd or Pd+). By generating fluorescence peaks through changes in the interaction of fluorescent signals with biomolecules, and based on the existence of interactions among these fluorescent molecules, it is possible to understand the structure and function of the molecule being detected by analyzing the composition of that molecule, thus enabling the exploration of specific functional pathways in biological cells. Such fluorescent markers are generally grown on the nanoscale, such as the different wavelengths of light found on the surface of biological samples and in ion channels. Their main regions of action include fluorescent effector moieties, surfactants, receptor-like cells, and some specific proteins in living organisms.

2.2. Fluorescent probes

Fluorescence probes, a fluorescence analysis method based on molecular recognition, use a specific interaction between subject and object to cause changes in the fluorescence properties of the system, thus selectively analyzing and identifying target compounds. Due to their high efficiency, sensitivity, and selectivity, fluorescent probes have been widely used for molecular, ion detection, and quantitative analysis in the field of analytical sciences [3].

There are fluorescent molecules with characteristic fluorescence in the Ultraviolet-Visible-near-IR Spectroscopy (UV-Vis-NIR) region. The fluorescence properties (excitation and emission wavelengths, intensity, lifetime, polarization, etc.) can change sensitively with the nature of the environment they are exposed to, such as polarity, refractive index, and viscosity. After being stimulated by light, the molecule returns from the stimulated singlet state to the ground state and emits characteristic light in the UV-vis-NIR region, which is called fluorescence. A class of fluorescent molecules whose fluorescence properties (excitation and emission wavelengths, intensity, lifetime, polarization, etc.) can change sensitively with the nature of the environment they are exposed to (e.g., polarity, refractive index, viscosity, etc.) are called fluorescent probes.

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3. Principle

3.1. Luminescence mechanism

The fluorescence signal of fluorescent markers is generated by the principle of light radiation based on the interaction of light with biological molecules. In general, the main mechanisms of luminescence of fluorescent probes are light-induced electron transfer, intramolecular charge transfer, proton transfer within the excited state, aggregated fluorescence enhancement, electron energy transfer, fluorescence resonance energy transfer, monomer-exciplex formation (Excimer-Exciplex. ME), rigidity effect, etc. [4].

The wavelength of light emitted by molecules is generally between 540 nm and 760 nm, and the wavelength of some luminescent substances changes. The longer the wavelength of light emitted, the higher the degree of fluorescence signal generation and the stronger the response to biomolecular signals. Therefore, it is necessary to utilize a large number of detected substances when performing light analysis. Since the interaction with biomolecules is much more complex, a more stable spectral range is required, which is important for trace molecule measurements. For example, when a compound is irradiated with infrared light, this compound may emit red, green, or blue radiation. Therefore these compounds can also be made into different instruments by modulating the intensity of light at different wavelengths. The most commonly used in the luminescence analysis of various trace molecules are Pd markers and Pd+ markers.

3.2. Probing molecular properties

Usually, the probe molecule reacts chemically with the probed substance and the resulting product molecules can exhibit different photophysical as well as photochemical properties Besides, the detection of the probed substance is achieved by characterizing the product molecules by fluorescence spectroscopy [5].

Substances with fluorescence properties often form specific complexes with certain metal ions (or anions) thus altering the original fluorescence signal (enhancement or burst), and therefore substances with fluorescence properties are often used to identify these ions with high selectivity [6]. Measuring whether a molecule has a specific physical property can be done by irradiating the sample with a laser. The purpose of laser irradiation is to characterize an unknown molecule using the intensity of laser-excited fluorescence. By measuring this molecule, it is possible to obtain what changes occur in that molecule when it is irradiated with laser light at this specific wavelength and how these changes change the size and molecular properties of that molecule. For biological samples, the specific properties include two aspects: one is the relationship between the intensity of the light signal that occurs throughout the body and the time it is exposed to light and the change in intensity; the other is the amount and structural characteristics of the substance in the organism calculated from the strength of the interaction between the light signal and the biological sample. For nano-scale biological samples, the relationship between the concentration of the substance and its energy change is more complex. Using nanotechnology to determine the concentration of substances in biological samples easily yields a material with different species, which is the nanomaterial effect.

In the field of biological samples, it is often necessary to detect the nature of biomolecules that may be present in biological samples by measuring the molecular weight at high content in the extracellular matrix. Nano-structural effect is generally suitable for detection of individual molecules in living organisms when there is no visible signal in the substance itself or when the intensity of the optical signal is very low.

3.3. Signal processing analysis

In addition to fluorescent signals, there are more complex phenomena that require specific signal processing analysis before reliable results can be obtained [7]. These methods of signal processing analysis include selective labeling of different molecules and selective modification of fluorescent materials. The methods of selective modification include photosensitization, ion chromatography, and

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disulfide bond modification. For proteins or compounds, their chemical properties and biological activities are different for specific structures and functions, so various factors must be considered when finding fluorescent markers with specificity (e.g., for Cu or Pd+) for selective modification. For example, for some proteins that do not have specific luminescence, selective modifications can generally be used to obtain better activeness [8]. Some substances are ones with strong biological effects; some substances have abiotic effects; some substances have biological effects which require more modification options for luminescent substances to achieve better imaging results. For biologically active substances such as receptors and antibodies, their photoconductive properties need to be changed to achieve better imaging analysis. Such receptor drugs may also have corresponding biological effects, which require rational selection of receptors and antibodies in the design; in addition, there are some substances that are toxic to organisms and need to ensure safety and effectiveness by means of metabolic control, which may affect diagnostic results or even lead to death.

4. Application

4.1. Spectrum

The main methods of spectroscopy based on fluorescence signals are spectral analysis, spectrometer analysis, nuclear magnetic resonance analysis, optical interferometric imaging, microscope scanning imaging, and fluorescence microscopy.

According to the spectral type, the light source can be divided into the diode light source (such as a fiber optic light source) and the monochromatic light source (such as a fluorescence microscope). For these two light sources, the monochromatic light source has the advantages of high spectral resolution, low spectral absorption, short spectral response time, and small change in the emission and reception wavelength of the light source. The monochromatic light source has good selectivity in the wavelength range, and at the same time, can obtain a spectral range that is long enough. It is currently recognized as a better monochromatic light source while the spectral response of its absorption peak is unstable. Because monochromatic light sources use a dispersion agent to regulate the interaction between two individual light-emitting units in the beam and the spectral response is influenced by the angle of the incident light. Monochromatic light sources are less affected by scattering intensity due to their longer wavelengths. Also, because monochromatic light sources have a high spectral response in the wavelength range, the light scattering imaging technique is a better imaging method for spectrometers. In addition, although light sources with longer wavelengths (e.g., 500 nm) also have higher sensitivity and higher image resolution in the wavelength range, they cannot be quantitatively detected due to their narrow wavelength range and are greatly limited by the intensity of the incident light by the light source, the refractive index of the lens, and other conditions. Therefore, it is generally believed that there is a certain difference in the quality of images obtained by different wavelength light sources, and there is also a certain connection with the nature of the material being measured.

4.2. Imaging

Biofluorescence imaging is a new approach to studying life phenomena using interactions between molecules. Biological probes of identical molecular weight and structure prepared using fluorescent markers for in vivo detection can provide additional information. In the field of biology, there have been many attempts by researchers to use fluorescent markers to study the properties of proteins, including whether there is a link between them and nucleic acids or products resulting from protein hydrolysis. Commonly used probes today include diatomic biofluorescent probes and polyatomic fluorescent probes, which can be used both as fluorescent markers and to study life phenomena. A growing number of known compounds and proteins have been discovered at the cellular and various molecular levels, many of which have been shown to be biologically active or potentially therapeutic, such as dioxins, multiple sclerosis, and tumors. All of them can be detected by bioimaging techniques. Fluorescent probes are enhanced when light waves are injected into cells or nanoparticles on the surface of a photocatalyst using luminescent proteins, and the photostimulation causes electrons in the excited photons to migrate,

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causing the photons to absorb more radiant energy from the sample. In many biological systems, such as cells and animals, there are proteins and chemicals with specific functions, the absence or overactivity of which can lead to changes in normal biological functions or even diseases.

4.3. Other applications

pH fluorescent probes are characterized by simple operation, high sensitivity, and high signal-to-noise ratio, especially when combined with laser confocal cell imaging. They have become an important tool for monitoring intracellular pH at the molecular level [9-11]. Lixia Cui et al. used indole (benzindole) derivatives and 4-hydroxybenzaldehyde as raw materials to synthesize semi-floral goodness dye compounds 1 and 2 in a one-step reaction, whose molecular structures contain two pH-sensitive sites of unsubstituted N atoms and phenolic hydroxyl groups on heterocycles, and then studied their spectral properties as pH probes using UV absorption and fluorescence spectroscopy [12].

Many substances undergo fluorescence changes in response to UV light. These are represented by molybdenum disulfide, tungsten disulfide, and gallium nitride; arsenic trioxide and lead dioxide; molybdenum disulfide and hexavalent vanadium; molybdenum disulfide and antimony trioxide; and titanium dioxide. These fluorescent probes have high resolution and sensitivity. For example, Griffin, Creste et al. used Taq-II molecules to detect a mixture of copper and zinc. Under UV irradiation, its fluorescence intensity increased with increasing light absorption by Cu and Zn and was proportional to the required detection time; in the wavelength range of 400 nm to 600 nm, the fluorescence intensity could be rapidly increased to more than 10 nm due to the strong penetrating power of UV light. The number of copper compounds, such as Cu and Zn determined by Creste in combination with UV light, also increased significantly.

5. Conclusion

This thesis is a preliminary study of the importance of fluorescent probes in biology, both in terms of their principles as well as their applications. In recent years, fluorescent molecular imaging techniques have developed rapidly and have been widely used to detect the composition, quantity, and strength of interaction with biomolecules in biological tissues. Fluorescent probes have received increasing attention due to their high sensitivity, specificity, and high throughput.

However, if this imaging technique is applied to disease diagnosis, more in-depth studies of fluorescent molecular markers will be needed. For example, there is a need to have accurate and clear imaging of a specific region within a cell, and thus more fluorescent signals must be obtained and a specific protein or molecule must be able to be accurately quantified. Therefore, many probes targeting biological tissues have now been gradually developed. For example, it has been found that unique fluorescent signals can be generated in certain proteins and precise localization within a certain range is achieved. However, in the case of specific functions, specific sites, or even metastases of cancer to other parts of the body, it is not yet possible to fully distinguish the time of action of different antigens or enzymes on the protein in the cell and the specific location of the enzyme in the body until the pathological test results are known or confirmed and then the relevant treatment is carried out to achieve precise treatment. Therefore, there is a need to develop microarrays that can accurately analyze cells and other components to make them more capable of imaging and facilitate clinical research tasks.

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