Design of Up-Conversion Fluorescence Imaging Device Based on Inverted Fluorescence Microscope

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Abstract. Fluorescence imaging is a widely used technique for microscopic observation in basic biomedical research. Currently, most of the commercial fluorescence microscopes are based on the design of down-conversion fluorescence reagent. In this paper, an up-conversion fluorescence imaging device based on inverted fluorescence microscope is designed. The device uses 980nm near-infrared as the excitation source and the inverted fluorescence microscope as the platform to transform its internal optical system and apply it to up-conversion fluorescence imaging. Through a large number of experimental studies, the stability and linearity of the equipment were verified, and the relationship between incubation time and intake of Hela cells was explored. The experimental results showed that when the laser power was 1.5W and UCNPs@ DSPE-PE concentration was 0.05mg/ml, the uptake of Hela cells reached the saturation state.

Introduction

Because many biomolecules are weakly fluorescent, external markers are often needed for more sensitive detection, and fluorescence detection is a more sensitive and effective technique. With the development of nanotechnology, upconverting fluorescent nanomaterials as a new generation of bioluminescent labels have advantages such as high chemical stability, low toxicity, high detection sensitivity, outstanding luminescence spectral characteristics, and large anti-Stokes shift[1]-[3]. Upconverting fluorescent nanoartials are currently widely used in biomedical, environmental testing, food safety and other fields[4]-[6]. In this paper, an up-conversion fluorescence imaging device based on inverted fluorescence microscope is studied, which realizes the detection of upconversion fluorescent nanomaterials and cell imaging. The designed device has the advantages of simple structure, low cost and stable performance.

Method

Detection Principle

Some substances emit luminescence when exposed to a specific wavelength and a certain intensity of light. When the light stops, the luminescence disappears. The emitted light is called fluorescence, and the substance that emits fluorescence is called fluorescent substance or fluorescent material. Usually these fluorescent signals are detected by photodetectors.

Set the signal strength received by the detector as $S$; then

$$ S = \frac{1}{2} \eta_{\text{PMT}} \Phi_F C_g \frac{8n_P(t)}{\text{pA}} $$

(1)
In the formula, $\eta_{PMT}$ is the fluorescence collection efficiency; $P(t)$ is the time averaged excitation light power; $C$ is the molecular density in the solution; $g$ is the time-averaged fluorescent signal; $\Phi_F$ is the fluorescence quantum yield.

$$\Phi_F = \frac{n_f - I_f}{n_e - I_f}$$  \hspace{1cm} (2)

In the formula, $n_f$ is the number of molecules that emit photons; $n_e$ is the number of molecules excited; $I_f$ is the fluorescence emission intensity; $I_a$ is the light intensity absorbed by the substance to be detected.

According to Bill-Lambert light absorption:

$$I_f = k I_0 (1 - 10^{\varepsilon L}) \Phi_f = k I_0 (1 - e^{-2.303 \varepsilon L}) \Phi_f$$  \hspace{1cm} (3)

In the formula, $\varepsilon$ is the molar extinction coefficient of the substance; $c$ is the concentration of the test substance; $L$ is the optical path length; In formula (2) and (3) the fluorescence intensity of the excitation and the concentration of the upconverting luminescent material can be regarded as a linear relationship, which is the theoretical basis for the quantitative detection of fluorescence [7], [8].

**Experimental Platform Construction**

The up-conversion fluorescence imaging device is mainly composed of excitation light path module, receiving light path module, extinction darkroom, PC, and inverted fluorescence microscope. The inverted fluorescence microscope adopts inverted fluorescence microscope (Jiangnan XD-202). The excited optical path module is composed of a laser and a binary component. The laser adopts MDL-III-980 fiber coupling laser (Changchun New Industries Optoelectronics Technology Co., Ltd). The binary component is composed of SMA-980nm fiber terminal collider, 45° optical adjustment frame, and a binary color filter (Edmund Optics® #69-208). Optical receiving routing is composed of digital camera, laser filter (Edmund Optics® #67-032) and extinction darkroom, as shown in figure 1.

The laser emitted by the laser passes through the collimator at the end of the optical fiber to convert the transmitted light in the optical fiber into collimator light. By finely adjusting the 45° optical adjustment frame, it is ensured that the 980nm laser projected upward can be con focal vertically through the microscope objective lens to the material to be tested in the petri dish. Meanwhile, the left extinction darkroom can ensure the leakage control of excitation light. Fluorescence generated by up-conversion fluorescent substances in the petri dish was collected by the objective lens and the residual excitation light was blocked by the laser filter through the detection of the range of excitation energy of the fluorophore. Finally, the image was collected by the photodetector. Figure 2 is the schematic diagram of the light path FIG. Figure 3 is a schematic diagram of a light path in which a red arrow represents an excitation light path and a green arrow represents a reception light path.

![Figure 1. Light path diagram.](image1)

![Figure 2. Light path diagram.](image2)

The detection light path adopts con focal mode. The light source is focused on the sample with dichotomy mirror and objective lens to stimulate the fluorescence signal. The fluorescence is
accepted by the photo detector through focusing lens and laser filter. At the same time, the extinction an echoic chamber can avoid stray light interference effectively and improve the signal-to-noise ratio of the detection system.

**Experimental Result**

After the upconversion fluorescence imaging device is designed, the stability and linearity of the instrument need to be tested. In order to analyze the linearity of the detection system, 7 samples with concentration of 0.0625mg/L, 0.125mg/L, 0.25 mg/L, 0.5 mg/L, 1mg/L, 2mg/L and 4mg/L were firstly configured. Set the power of the 980nm laser to 1.5w and finally convert the obtained fluorescence signal of up-converted sample solution into fluorescence image through the photoelectric detector, and calculate the average value of R and G components of all pixel points. Figure 3 (a) and (b) test results were obtained, Figure 3 (a) is the relationship between sample concentration and the mean value of G component, and figure 3(b) is the relationship between sample concentration and the mean value of R component. Through the analysis of the test results of different concentrations of samples, it is known that there is a good linear relationship between the fluorescence intensity and the logarithm of the sample concentration.

In order to evaluate the stability of the device designed in this paper. First, the hela cells were incubated with UCNPs@ dspe-peg with concentrations of 0.1mg/L, 0.2mg/L and 0.3mg/L respectively for 1 hour. Thereafter, it was continuously detected 30 times every 60 seconds. The test results are shown in Figure 4. It can be seen that the data fluctuation of the detected fluorescence intensity is substantially on a horizontal line and the fluctuation decreases as the concentration of the up-converted sample solution decreases. In the experiment, it was verified that the detection system had good stability by analyzing the test results of the same sample which were continuously tested for 30 times every 60s.

![Figure 3](image3.png)  
**Figure 3.** Upconversion imaging device linearity.

![Figure 4](image4.png)  
**Figure 4.** Stability of upconversion imaging device.
If the laser power is too low, it will be difficult to excite low-concentration up-conversion samples. When the power is too high, it will cause noise. Therefore this study uses 980nm near-infrared as the excitation source so that the imaging device can not only effectively excite the up-conversion sample solution to emit fluorescence signals but also not cause strong noise interference. In this experiment, the HeLa cells were first incubated with 0. 1mg/mL UCNPs@DSPE-PEG, 0. 2mg/mL UCNPs@DSPE-PEG, 0. 4mg/mL UCNPs@DSPE-PEG for 1 hour, and then the power was adjusted to 2. 0W, 1. 5W, 1. 0W and 0. 5W, and the Jiangnan microscope HDCE-X5 camera was used for shooting. The result is shown in figure 5. Considering effective sample excitation and avoiding unnecessary noise generation, 1. 5W was finally selected as the excitation power of the imaging device.

After selecting the appropriate excitation power, further explore the intake of hela cells to different concentrations of UCNPs@DSPE-PE. Firstly, the hela cells and their concentrations were respectively 0. 025mg/mL UCNPs@DSPE-PE, 0. 05mg/mL UCNPs@DSPE-PE, 0. 1mg/mL UCNPs@DSPE-PE, 0. 2mg/mL UCNPs@DSPE-PE and 0. 4mg/mL UCNPs@DSPE-PEe for incubation for one hour for fluorescence imaging. We used a designed up-conversion fluorescence imaging device to image the hela cells. Stimulated by near-infrared 980nm excitation light, hela cells produce fluorescence that is detected by a detector and then imprinted by an imaging device. The specific fluorescence microscope images are shown in figure 7. The figure shows the fluorescence images of hela cells at 1. 5W with different incubation concentrations under the condition of incubation for 1 hour, bright field and dark field. It can be seen from the figure that the concentration reaches the saturation state at 0. 05mg/ml. With the increase of concentration, the phagocytosis of cells will not be increased and the material remaining in the solution will also be excited. Therefore, the most suitable concentration is 0. 05mg/ml.

Figure 5. To explore the influence of power on the intake of hela cells.

Figure 6. The relationship between UCNPs@ dspe-pe concentration and intake.
Summary

In order to meet the current market demand for up-conversion imaging devices, the common inverted fluorescence microscope was modified and applied to cell imaging. By analyzing the results of sample detection with different concentrations, it is verified that the fluorescence intensity has a good linear relationship with the logarithm of sample concentration. At the same time, the relationship between the concentration of UCNPs@ dspe-pe and the intake was explored. The experimental results showed that when the laser power was 1.5w and the concentration of UCNPs@ dspe-pe was 0.05mg/ml, the intake of hela cells reached the saturation state. With the increase of the concentration, the phagocytosis of cells would not be increased and the remaining materials in the solution would also be excite.

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