

## Article

# Upconversion Nanoparticle-Based Luminescence DNA Sensor on Porous Silicon Substrate

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## Abstract

Rare-earth-doped upconversion nanoparticles (UCNPs) exhibit upconversion luminescence upon excitation with infrared light and have been extensively utilized in the field of biosensing. In this study, a UCNPs-based biosensor with porous silicon (PSi) as the substrate was developed for the first time, enabling the detection of target DNA molecule concentration. First, a PSi substrate was prepared via electrochemical etching and subsequently functionalized to enable target DNA molecules to immobilize onto the inner walls of the PSi substrate's pores. Then, UCNPs-labeled probe DNA molecules hybridized with the target DNA molecules, enabling indirect attachment of UCNPs to the inner walls of the PSi substrate. Subsequently, the sample surface is irradiated with a 980 nm laser. Upconversion fluorescence images of the sample, both before and after the biological reaction, are captured using an image acquisition device. Image processing software is employed to calculate the average change in grayscale values, enabling the determination of the molecular concentration of target DNA. The limit of detection (LOD) of this method for target DNA molecular concentration is 86 pM, demonstrating that it enables low-cost, highly sensitive, rapid, and convenient biological detection of target DNA molecules.

**Keywords:** upconversion nanoparticle; DNA; porous silicon; grayscale values

## 1. Introduction

UCNPs are a class of novel fluorescent labeling materials that absorb long-wavelength near-infrared photons and emit short-wavelength ultraviolet-visible photons. Near-infrared-excited UCNPs offer numerous advantages, including minimal tissue damage, strong tissue penetration, absence of background fluorescence interference, and no photobleaching effect. Consequently, they have found extensive applications in fields such as biosensors [1–3] and bioimaging [4]. In addition to UCNPs, numerous nanomaterials have been applied in luminescent biosensors. For instance, metallic nanostructures like AuNPs and AgNCs are valued for their outstanding quenching efficiency and surface-enhanced fluorescence properties, while quantum dots (QDs) are noted for their high brightness and broad excitation spectra. Nevertheless, QDs are often limited by potential toxicity and persistent photobleaching. Additionally, graphene oxide (GO) serves as a versatile energy-transfer scaffold for “turn-on” sensing [5]. A common drawback of these conventional fluorophores is their reliance on short-wavelength excitation, which typically induces background autofluorescence from biological samples and potential phototoxicity. UCNPs overcome these limitations by converting near-infrared (NIR) light excitation into



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visible emission, providing a background-free signal that substantially improves sensing sensitivity within complex biological matrices. To date, numerous scholars have published articles on the application of UCNPs in biosensing technologies. For example, Liu et al. [6] adopted  $\text{NaYF}_4:\text{Yb,Tm}@\text{NaYF}_4$  core-shell structured UCNPs to establish a label-free photo-electrochemical platform, realizing highly specific quantitative detection of target DNA. Yan et al. [7] exploited  $\text{UCNPs}@\text{SiO}_2@\text{CeO}_2$  core-shell-shell multifunctional nanocomposites and combined them with CRISPR-Cas12a signal amplification technology, thus developing a dual-mode upconversion luminescence/colorimetric detection method for nucleic acids. To date, no reports have been published on upconversion fluorescence studies based on UCNPs in PSi or their applications in bioassays.

As a nanostructured material, PSi has been widely applied in the field of biosensors due to its excellent properties, including a large specific surface area, good biocompatibility, controllable pore sizes, and facile functionalization [8–11]. To date, optical PSi-based biosensors have been predominantly classified into two modalities: detection based on fluorescence changes [12–14] and refractive index changes [15,16]. Among these, fluorescence-based biosensors utilize the fluorescence emitted by excited semiconductor or graphene quantum dots for detection. They exhibit high sensitivity, excellent selectivity, minimal required biomaterial, rapid response times, and good repeatability. However, since the excitation of conventional quantum dot fluorescence requires short-wavelength light (typically less than 480 nm) or even ultraviolet (UV) light, this exerts a certain impact on the biomolecules being detected. Additionally, due to the strong absorption of short-wavelength light by PSi materials, the excitation efficiency of short-wavelength light on fluorescent particles that label bioprobe molecules within PSi mesopores is reduced. By employing near-infrared excitation light, which exhibits weaker absorption within PSi, fluorescent particles that label bioprobe molecules in PSi pores can be more effectively excited. Moreover, the optical penetration window of biological tissues lies in the near-infrared region. UCNPs were adopted as fluorescent labels for two key advantages. On one hand, NIR light exhibits weak absorption in PSi, enabling more efficient excitation of the fluorescent nanoparticles conjugated to bioprobe molecules within PSi pores. On the other hand, the optical transparency window of biological tissues lies in the NIR region. Therefore, the utilization of NIR excitation light minimizes photodamage to biomolecules [17] and it also avoids the interference to biological detection caused by the autofluorescence emitted by many organisms and their tissues under visible light excitation [18]. In summary, the application of UCNPs as fluorescent labels on PSi substrates represents a highly promising strategy, and to the best of our knowledge, no relevant research has been reported to date.

The vast majority of currently reported UCNP-based biosensors employ a “turn-on” detection mechanism, wherein fluorescence quenching occurs through FRET between the UCNPs and fluorescent quenchers [19–21]. The introduction of fluorescent quenchers not only increases the complexity of sensor construction but also poses challenges for PSi substrates, as the large size of quencher molecules hinders their entry into PSi pores. To address this issue, a novel upconversion fluorescent bio-detection method based on PSi is proposed, which operates without the need for fluorescent quenchers.

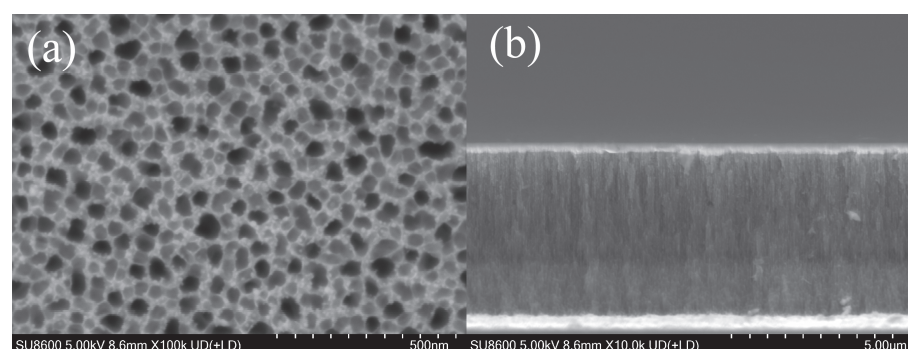
In this study, a novel upconversion fluorescent bio-detection method based on a PSi substrate is proposed. Compared with previous methods using QDs as fluorescent labels [22], which rely on short-wavelength excitation that may cause photodamage to biological samples and suffer from reduced excitation efficiency due to the strong absorption of short-wavelength light by the PSi material, our approach utilizes UCNPs to overcome these limitations. First, a PSi substrate was fabricated via anodic electrochemical etching. Subsequently, the substrate was functionalized to immobilize target DNA molecules on the inner walls of its pores. UCNPs are conjugated with probe DNA molecules to serve as

upconversion fluorescent probes. These probe molecules react with target DNA molecules in the pores of the PSi substrate, enabling indirect linkage between the UCNPs and the PSi substrate. Using a 980 nm laser to excite the UCNPs within the pores of the PSi substrate, an image acquisition device captured upconversion fluorescence images through a 650 nm bandpass filter. Subsequently, image processing software calculated the average gray value of the images, and the change in the average gray value before and after the reaction was further computed. Since probe DNA molecules are conjugated with UCNPs, the number of UCNPs reflects the quantity of target DNA molecules. That is, the variation in image gray value can reflect the concentration of target DNA molecules. Detection of the concentration of target DNA molecules can be achieved by establishing the relationship between the variation in average gray value and the concentration of target DNA molecules.

## 2. Materials and Methods

### 2.1. Preparation of the PSi Substrate

P-type single-crystal silicon wafers ( $\langle 100 \rangle$ , boron-doped,  $0.01\text{--}0.05\ \Omega\cdot\text{cm}$ ,  $400 \pm 25\ \mu\text{m}$  thick) were purchased from Shandong Yuanjing Electronics Technology Co., Ltd., (Jinan, China) and cut into  $1.5\ \text{cm} \times 1.5\ \text{cm}$  small pieces. The PSi region to be prepared was round with a diameter of 0.8 cm. The small pieces were sequentially placed into acetone (Chengdu Kelong Chemical Co., Ltd., Chengdu, China), ethanol (Tianjin Xinbote Chemical Co., Ltd., Tianjin, China), and deionized water (DI water), and ultrasonically cleaned for 10 min in each solution to remove impurities on the surface of the silicon pieces. The silicon pieces were placed into a home-made polytetrafluoroethylene (PTFE) etching cell. The etching apparatus employed a two-electrode configuration to ensure a stable electrochemical reaction: a copper (Cu) plate was used as the anode contact, pressed firmly against the backside of the silicon wafer to ensure uniform current distribution, while a copper (Cu) ring served as the cathode immersed in the electrolyte [23]. Absolute ethanol and hydrofluoric acid (HF, Tianjin Xinbote Chemical Co., Ltd., Tianjin, China) at a volume ratio of 1:1 were poured into the etching cell, and the anodization conditions were controlled via the LabVIEW (8.2, National Instruments, Austin, TX, USA) program. To ensure that UCNPs could penetrate smoothly into the pores of the PSi substrates, the PSi substrates with a pore size of 50 nm and a PSi layer thickness of  $5\ \mu\text{m}$  were successfully fabricated via electrochemical etching under a current density of  $120\ \text{mA}/\text{cm}^2$  for 30 s. After etching, the PSi substrate was taken out, rinsed with absolute ethanol and DI water, and dried in nitrogen. The surface and cross-sectional SEM images of the PSi substrate are shown in Figure 1.



**Figure 1.** SEM images of the PSi substrate: (a) surface SEM image; (b) cross-sectional SEM image.

### 2.2. Functionalization of PSi Substrates

To enable target DNA molecules to conjugate to the inner walls of the pores of the PSi substrate, a series of modifications is required for the PSi substrate, namely oxidation, silanization, and glutaraldehyde treatment. Freshly prepared PSi substrates exhibit high

instability and are highly prone to oxidation; thus, oxidation treatment is required to stabilize their properties. The PSi substrates were immersed in a 30% hydrogen peroxide solution ( $\text{H}_2\text{O}_2$ , Chengdu Kelong Chemical Co., Ltd., Chengdu, China), placed in an oven at 60 °C, and oxidized for 3 h. After oxidation, they were rinsed with absolute ethanol and DI water and dried in nitrogen. Subsequently, the PSi substrates were silanized: the oxidized PSi substrates were immersed in a 4% 3-aminopropyltriethoxysilane solution (volume ratio of APTES:methanol:DI water = 1:10:10; both APTES and methanol were purchased from Aladdin Biochemical Technology Co., Ltd., Shanghai, China) for 1 h, then rinsed with absolute ethanol and DI water, and dried in nitrogen—this successfully conjugated APTES molecules to the pore surfaces of the PSi substrates. To form stable amino groups on the pore surfaces of the PSi substrates, the samples were placed in a vacuum oven at 100 °C and heated for 10 min [24], then removed and allowed to cool naturally. Next, the PSi substrates were subjected to glutaraldehyde treatment: the silanized PSi substrates were immersed in a 2.5% glutaraldehyde aqueous solution (volume ratio of 50% glutaraldehyde aqueous solution (Hubei Bizion Biological Chemical Co., Ltd., Wuhan, China): DI water = 1:19) for 1 h, then rinsed with phosphate-buffered saline (PBS, pH 7.4) and DI water, and dried in nitrogen.

### 2.3. Immobilization of Target DNA Molecules on Pore Walls of PSi Substrates

The DNA molecules used in this work were purchased from Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China. Their specific sequences are as follows:

Target DNA molecule: 5'-CGCGGCCTATAAGCTTGTTG-3'-NH<sub>2</sub>

Probe DNA molecule: 5'-cAACAAGCTTATAGGCCGCG-3'-NH<sub>2</sub>

The DNA molecules are 20 bases in length, with each base approximately 0.34 nm long. Based on the calculation, the length of the DNA molecules used in this work is approximately 6 nm. The target DNA molecules were diluted with PBS to seven concentrations: 5 µM, 1 µM, 100 nM, 10 nM, 1 nM, 0.5 nM, and 0.1 nM. Aliquots of 40 µL of the target DNA molecules at each of these seven concentrations were separately pipetted onto the surface of the functionalized PSi substrates. The substrates were then incubated in a 37 °C incubator for 2 h to allow the target DNA molecules to bind to the pore walls of the PSi substrates. Subsequently, they were rinsed with PBS and dried in nitrogen. Subsequently, the samples were immersed in a 3 M ethanolamine hydrochloride solution and again incubated in a 37 °C incubator for 2 h. After removal, they were rinsed with PBS and dried in nitrogen. This step was performed to block the aldehyde groups that had not reacted with the target DNA molecules, preventing the probe DNA molecules from binding to these unreacted aldehyde groups and thereby avoiding interference with subsequent steps.

### 2.4. Conjugation of UCNPs with Probe DNA Molecules

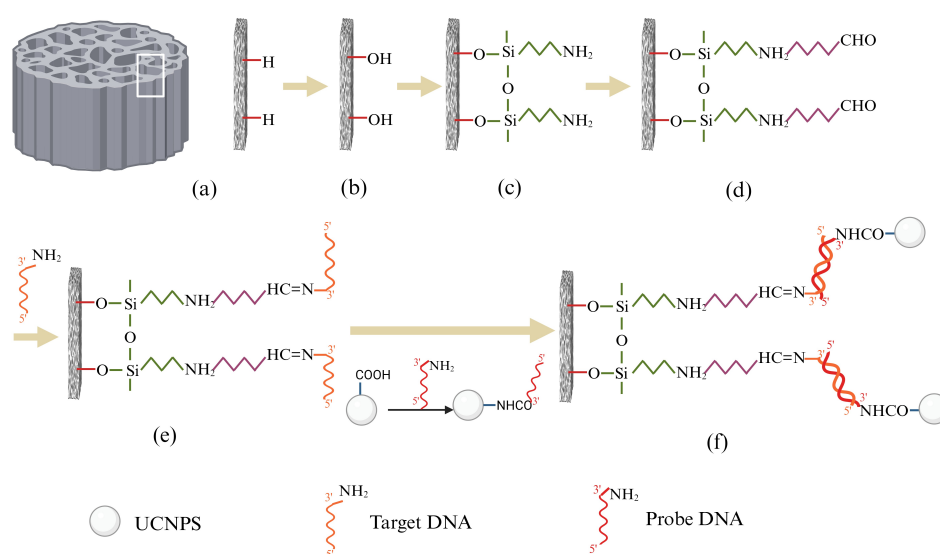
The UCNPs used in this study are PAA-modified NaErF<sub>4</sub>: Yb/Tm@NaYF<sub>4</sub>, purchased from Hangzhou Xinqiao Biotechnology Co., Ltd., Hangzhou, China. They have a diameter of approximately 12 nm, are excited at 980 nm, and exhibit a fluorescence peak at 650 nm. A TEM image of these UCNPs is shown in Figure S1 of the Supporting Material. To enable successful conjugation of UCNPs with probe DNA molecules, 50 µL of UCNPs (4 mg/mL) were first added to 90 µL of PBS and vortexed for 10 min. This was followed by the addition of 30 µL of 0.01 M 1-ethyl-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Aladdin Biochemical Technology Co., Ltd., Shanghai, China) solution and 30 µL of 0.01 M N-hydroxysulfosuccinimide sodium salt (NHS, Aladdin Biochemical Technology Co., Ltd., Shanghai, China) solution; the mixture was vortexed for 10 min to allow the reaction to proceed, thereby activating the carboxyl groups. Subsequently, 200 µL of probe DNA molecules (20 µM) were added, and the mixture was vortexed for 10 h under light-

protected conditions to ensure sufficient conjugation between UCNPs and probe DNA molecules. The mixture was then centrifuged at 10,000 rpm for 10 min; the supernatant was discarded to remove uncoupled probe DNA molecules, and the precipitate was retained. The precipitate was resuspended in 400  $\mu$ L of PBS, yielding UCNPs-labeled probe DNA molecules (UCNPs-pDNA).

## 2.5. Hybridization of Target DNA Molecules with UCNPs-pDNA Molecules

50  $\mu$ L of UCNPs-pDNA was pipetted onto the surface of PSi substrates with immobilized target DNA molecules. The substrates were then incubated in a 37 °C incubator for 2 h to allow sufficient reaction. After removal, they were rinsed with PBS to remove unhybridized UCNPs-pDNA molecules (those not hybridized with target DNA molecules) and dried in nitrogen. Through Watson-Crick base complementary pairing, the target DNA molecules and probe DNA molecules formed stable double-stranded DNA (dsDNA) complexes, which enabled the indirect immobilization of UCNPs on the pore surface of PSi. Each process of PSi functionalization and the principle of the biological reaction are illustrated in Figure 2.

Specifically, Figure 2: (a) shows freshly prepared PSi; (b) depicts oxidized PSi; (c) illustrates silanized PSi; (d) presents glutaraldehyde-modified PSi; (e) demonstrates the conjugation of target DNA molecules to the pore walls of PSi; (f) reveals the hybridization between UCNPs-pDNA molecules and target DNA molecules.

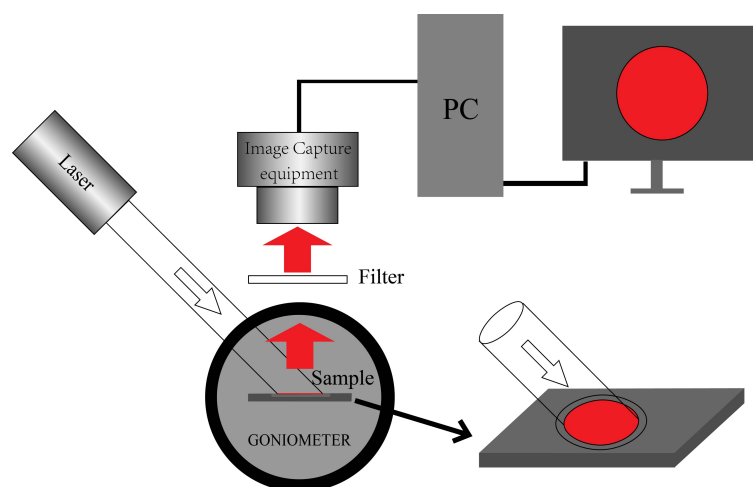


**Figure 2.** Schematic diagram of PSi functionalization and biological reactions. (a) Fresh PSi; (b) Oxidized PSi; (c) Silanized PSi; (d) Glutaraldehyde-modified PSi; (e) Target DNA molecules immobilized on the PSi surface; (f) Hybridization between UCNPs-pDNA molecules and target DNA molecules.

## 2.6. Detection of Biological Reactions

In this study, the detection of biological reactions is achieved by comparing the change in the average gray value of the images before and after the biological reaction. After the biological reaction, within the pores of the PSi substrate, probe DNA molecules undergo a hybridization reaction with target DNA molecules, thereby enabling UCNPs to be indirectly attached to the pore walls of the PSi substrate via probe DNA molecules. Upon irradiation with a 980 nm infrared laser, UCNPs within the pores emit 650 nm upconversion fluorescence. This fluorescence is emitted through the surface of the PSi substrate, forming a red image. By detecting the change in the average gray value of the upconversion fluorescence images before and after the biological reaction, the concentration of target DNA molecules can be determined. The biological reaction detection system is shown in Figure 3.

In Figure 3, the laser used is a 980 nm laser (MDL-III-980, Changchun New Industries Optoelectronics Technology Co., Ltd., Changchun, China). The filter employed is a 650 nm bandpass filter (BP650, Shenzhen Nahong Optoelectronics Technology Co., Ltd., Shenzhen, China), which allows only light with a wavelength of 650 nm to pass through. The image acquisition device is an IQOO 9 Pro smartphone (IQOO Digital Co., Ltd., Dongguan, China). The light emitted by the laser irradiates the surface of the sample to be tested at an oblique angle of 45 degrees from a distance of 5 cm, exciting UCNPs to generate upconversion fluorescence. The upconversion fluorescence passes through the filter and enters the image acquisition device, resulting in upconversion fluorescence images, and the average gray value of the upconversion fluorescence images is obtained via a computer. The entire detection process of upconversion fluorescence images before and after the biological reaction is conducted in a completely dark environment, eliminating the influence of stray light.

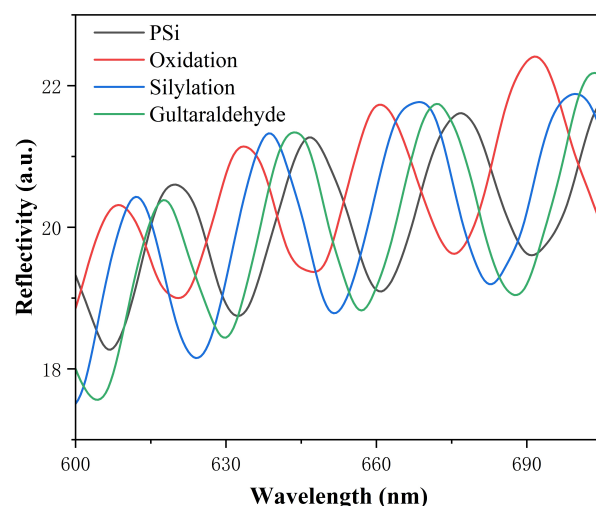


**Figure 3.** Biological reaction detection device.

### 3. Results and Discussion

#### 3.1. Changes in Reflectance Spectra During the Functionalization Process

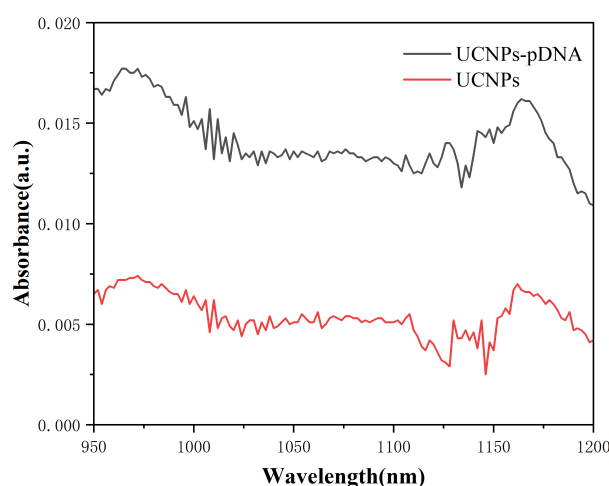
The steps of the functionalization process—oxidation, silanization, and glutaraldehyde treatment—all induce changes in the reflectance spectra of the substrate. Thus, whether functionalization is successful can be determined by means of the reflectance spectra of the sample [25]. Figure 4 shows the reflectance spectra of the substrate after each step of the functionalization process. The reflectance spectra were measured using a Hitachi U-4100 UV-Vis-NIR spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan). The black line represents the reflectance spectrum of the freshly prepared PSi substrate; the red line represents that of the substrate after oxidation; the blue line represents that of the substrate after silanization; and the green line represents that of the substrate after glutaraldehyde treatment. The oxidation process leads to the formation of SiO<sub>2</sub> on the surface of the PSi substrate. The refractive index of only 1.45 of SiO<sub>2</sub> is much lower than that of monocrystalline silicon (3.4), causing a blue shift in the reflectance spectrum. Subsequent silanization and glutaraldehyde treatment result in molecules attaching to the pore walls of the substrate, increasing the effective refractive index of the substrate and inducing a red shift in the sample's reflectance spectrum. The shift in the reflectance spectrum of the PSi substrate in Figure 4 indicates that each step of functionalization is successful.



**Figure 4.** Changes in reflectance spectra of PSi substrates during functionalization. The black line represents freshly prepared PSi; the red line represents that of PSi after oxidation; the blue line represents that of PSi after silanization; and the green line represents that of PSi after glutaraldehyde treatment.

### 3.2. Conjugation Characterization of UCNPs with Probe DNA Molecules

Figure 5 shows the absorption spectra of UCNPs and UCNPs-pDNA, which were recorded using a UV-3600 UI-Vis-NIR spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The samples were dispersed in PBS buffer at a concentration of 0.15 mg/mL, and measurements were conducted using a 1 cm pathlength quartz cuvette. The red curve represents the absorption spectrum of pristine UCNPs, while the black curve corresponds to that of UCNPs conjugated with probe DNA molecules. It can be observed that after conjugation with probe DNA molecules, the characteristic absorption peaks of UCNPs exhibited a distinct redshift, with their positions shifting from 962 nm and 1160 nm to 966 nm and 1164 nm, respectively. This result indicates the successful conjugation between UCNPs and probe DNA molecules.

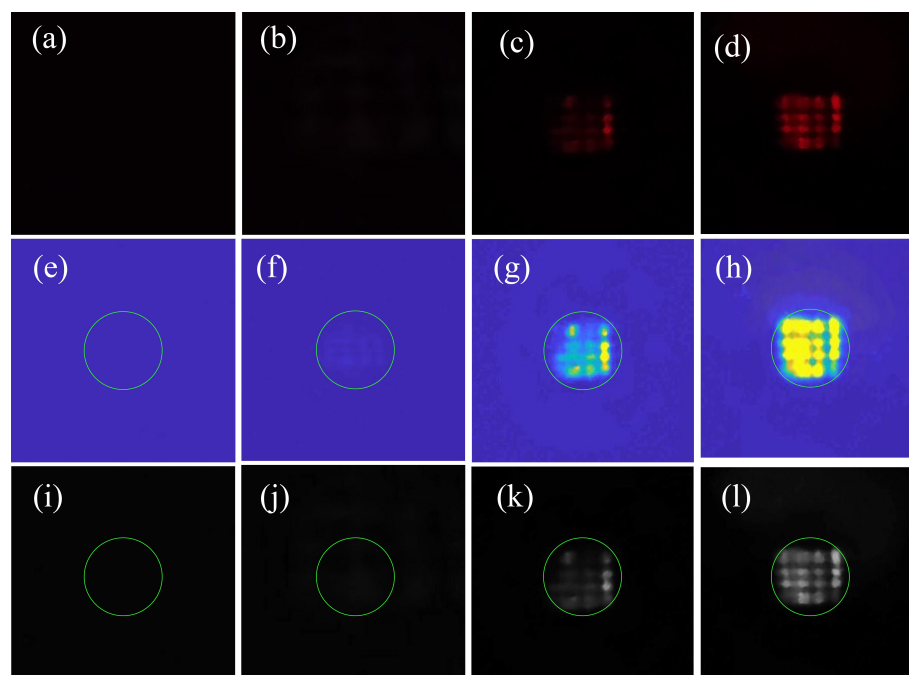


**Figure 5.** Absorption spectra of UCNPs and UCNPs-pDNA. The red line represents the absorption spectrum of UCNPs; the black line represents that of UCNPs-pDNA.

### 3.3. Detection Results of Biological Reactions

In Figure 6, (a)–(d) display the upconversion fluorescence images of the PSi substrate after the reaction with probe molecules at target DNA concentrations of 0 nM, 10 nM, 100 nM and 1  $\mu$ M, respectively. (e)–(h) correspond to the pseudocolor images of (a)–(d), respectively. (i)–(l) correspond to the grayscale images of (a)–(d), respectively. The selected

green circular region fully covers the entire laser spot irradiated on the PSi substrate surface, and the average gray value was calculated from all pixel points within this region. When the concentration of target DNA molecules is low, the upconversion fluorescence intensity is weak, making it difficult to observe changes with the naked eye; however, significant changes in the average gray value of the upconversion fluorescence images can be detected using image processing software. Since probe DNA molecules are conjugated with UCNP, the number of UCNP reflects the number of target DNA molecules. As the concentration of target DNA increases, the number of UCNP-pDNA molecules reacting with target DNA molecules also increases, and the upconversion fluorescence intensity of UCNP increases accordingly.



**Figure 6.** The upconversion fluorescence images and their corresponding pseudocolor images on the surface of the PSi substrate. (a–d) are the upconversion fluorescence images after the reaction of probe molecules with target DNA molecules at concentrations of 0 nM, 10 nM, 100 nM and 1  $\mu$ M on the PSi substrate, respectively. (e–h) are the corresponding pseudocolor images of (a–d), respectively. (i–l) are the corresponding grayscale images of (a–d), respectively.

Figure 7a is a plot of the relationship between different concentrations of target DNA molecules in the range of 0.1 nM to 5  $\mu$ M and their corresponding changes in the average gray value of upconversion fluorescence images. It can be seen that within this range, there is a good logarithmic relationship between the target DNA concentration and the change in average gray value, and the logarithmic equation is:

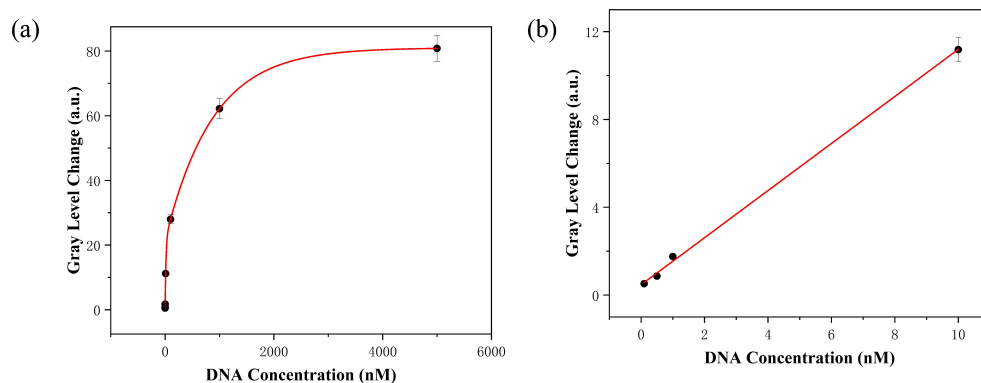
$$Y = 81.01 - 21.29e^{-\left(\frac{X}{139}\right)} - 43.43e^{-\left(\frac{X}{80.39}\right)} \quad (1)$$

In Equation (1), Y denotes the change in average gray value of fluorescence images, X denotes the concentration of target DNA molecules, and the fitting coefficient is 0.999.

Additionally, in the concentration range of 0.1 nM to 10 nM, there is a good linear relationship between the concentration of target DNA molecules and the change in average gray value of upconversion fluorescence images, as shown in Figure 7b, and the linear equation is:

$$Y = 0.47 + 1.07X \quad (2)$$

In Equation (2),  $Y$  denotes the change in average gray value of upconversion fluorescence images,  $X$  denotes the concentration of target DNA molecules, and the fitting coefficient is 0.999. The LOD was calculated as 86 pM ( $\text{LOD} = 3\sigma/s$  [26], where  $\sigma$  is the standard deviation of ten consecutive measurements of blank samples, and  $s$  is the slope of the linear equation).



**Figure 7.** Correlation between different target DNA concentrations and the corresponding changes in the average gray value of fluorescence images before and after the biohybridization reaction, with the curve representing the fitted regression line. (a) 0.1 nM–5  $\mu$ M; (b) 0.1 nM–10 nM.

### 3.4. Reproducibility and Stability

Stability and repeatability of sensor detection are key factors for achieving target detection. In this experiment, 10 PSi substrates were fabricated under the same conditions, with the concentration of target DNA used being 100 nM. The average gray value of fluorescence images of the 10 samples was detected, and the relative standard deviation (RSD) of the detection results was 2.70%. Subsequently, the average gray value of the samples was measured every 5 days, for a total of four measurements, with the changes in gray value being 28.00, 27.41, 27.01, and 26.85, respectively. The minor decrease in grey values observed over 15 days might be due to the slight desorption of UCNP or the natural degradation of DNA molecules. However, the sensor maintained over 95% of its original signal, demonstrating acceptable long-term stability. These results confirm that the DNA sensor fabricated in this study exhibits excellent stability and repeatability.

### 3.5. Advantages of Sensors Fabricated in This Study

Compared with traditional sensors based on the upconversion nanoparticle (UCNP)-fluorescent quencher “turn-on” sensing strategy, the DNA sensor fabricated in this work eliminates the need for fluorescent quenchers, featuring a simpler configuration and easier preparation process. In addition, the sensitivity of the sensor prepared by this method is significantly superior to that of several existing methods based on the refractive index variation of porous silicon substrates. For comparison, the LOD of the conventional label-free reflectance spectroscopy sensor reaches 21.3 nM [27]. Even when CdSe/ZnS QDs are used to label target molecules for refractive index signal enhancement, its LOD only achieves 6.97 nM [28]. Furthermore, the LOD of a label-free angular spectroscopy method that does not require a spectrometer is as high as 42 nM [29]. Although the LOD of the proposed method is slightly higher than that of the fluorescence spectroscopy-based method (10 pM) [30], the fluorescence image acquisition for a single sample takes only a few seconds. In contrast, both reflectance spectrometers and fluorescence spectrometers require tens of seconds to complete the scanning of one sample, indicating that our method is much more rapid. Moreover, the exclusion of spectrometers greatly reduces the overall detection cost.

## 4. Conclusions

In this study, a PSi substrate with a pore size of 50 nm was obtained via anodic electrochemical etching. By conjugating UCNP with probe DNA molecules, an upconversion fluorescent probe molecule was prepared. This probe molecule reacts with target DNA molecules immobilized in the pore channels of the PSi substrate, enabling UCNP to be indirectly attached to the pore channels of the PSi substrate. When the surface of the PSi substrate is irradiated with a 980 nm laser, UCNP emits 650 nm upconversion fluorescence. Upconversion fluorescence images of the samples are acquired using image acquisition equipment, and the change in average gray value of the upconversion fluorescence images before and after the reaction is derived using image processing software. Subsequently, the relationship between the concentration of target DNA molecules and the change in their average gray value is established, enabling the detection of target DNA molecule concentration. The sensor proposed in this study has a LOD of 86 pM, exhibiting high detection sensitivity and achieving low-cost, convenient, and rapid biological detection.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/bios16020105/s1>, Figure S1: TEM image of UCNP.

**Author Contributions:** Conceptualization, Z.J. and Y.L.; methodology, Z.J. and Y.L.; investigation, Y.Z. and Z.Y.; resources, Y.L., X.H. and J.W.; data curation, Y.Z. and X.W.; formal analysis, Y.Z., J.W., X.W. and Z.Y.; writing—original draft, Y.Z. and X.W.; supervision, X.H. and J.W.; writing—review and editing, Z.J. and Y.L.; funding acquisition, Z.J. and J.W.; software, X.H. and J.W.; validation, Y.Z., X.H. and J.W.; visualization, Y.Z., X.W. and Z.Y. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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