FAST AND HIGHLY SENSITIVE LASER SCANNER FOR RECORDING PHOTON-UPCONVERSION LUMINESCENCE FROM PLANAR SURFACES

Antonín HLAVÁČEK, Jana KŘIVÁNKOVÁ, František FORET

Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic, EU,
hlavacek@iach.cz

Abstract

Photon-upconversion nanoparticles (UCNPs) are lanthanide-doped nanocrystals that can be excited by near-infrared light and emit photon-upconversion luminescence of shorter wavelengths. Advantages of UCNPs include near-infrared excitation, multiple and narrow emission bands, negligible autofluorescence and high stability, which make UCNPs ideal luminescence label for use in biological and chemical assays. These assays - e.g. upconversion-linked immunosorbent assay, western blot, lateral flow assay, gel electrophoresis, thin layer chromatography - commonly require the scanning of a planar surface with a high spatial resolution and an excellent sensitivity. The availability of commercial equipment is recently limited because of the novelty of the photon-upconversion phenomenon. Therefore, we report on the construction of photon-upconversion laser scanner. The scanner consists of a laser scanning head, which is attached to a xy-moving stage. The scanning head itself is constructed as an epiluminescence detector with excitation wavelength of 976 nm. A CCD array spectroscope is connected to the laser head and serves as a sensitive detector of photon-upconversion luminescence. The scanner possesses a spatial resolution of 200 µm, the scanning rate is up to 57 points per second and the sensitivity reaches down to single photon-upconversion nanoparticle.

Keywords: Photon-upconversion, nanoparticle, laser scanner, hyperspectral imaging

1. INTRODUCTION

Multiplexed assays are suitable for parallel analyte detection. Even in complex mixtures, multiplexed detection reduces the consumption of sample, reagents, and other resources [1-5]. The applications of multiplexed assays range from single-cell [6] and single-molecule assays [7], medical diagnostics [1], detecting biomolecule interactions [8], environmental monitoring [9], pathogen detection [10], multiparameter chemical and biological assays [11] and screening of chemical libraries [12].

Photon-upconversion nanoparticles (UCNPs) are luminescence labels, which are widely considered as a vital alternative to fluorescence reporter in multiplexed assays [13-15]. UCNPs are lanthanide-doped nanocrystals that can be excited by near-infrared light and emit photon-upconversion luminescence of shorter wavelengths [16-18]. Advantages of photon-upconversion nanoparticles include near-infrared excitation, multiple and narrow emission bands, negligible autofluorescence and high stability. The assays with UCNPs - e.g. upconversion-linked immunosorbent assay, western blot, lateral flow assay, gel electrophoresis, thin layer chromatography - commonly require the scanning of planar surfaces with a high spatial resolution and an excellent sensitivity. Currently reported equipment (photon upconversion scanners) only allows for reading the sum of intensities in a single spectral range [19].

Here, we address this limitation and introduce a hyperspectral photon-upconversion laser scanner. The laboratory-made scanner consists of an epiluminescence laser scanning head, which is attached to a xy-moving stage. A CCD array spectroscope is connected to the laser head and serves as a sensitive detector of photon-upconversion luminescence. We provide the comparison of scanning for three types of nanoparticles containing either Ho³⁺, Er³⁺ or Tm³⁺ as activator ions. We also show the capability of scanner to record emission spectrum from a single UCNP.
2. MATERIAL AND METHODS

2.1. Scanner construction and control

The scanner was composed of two modular parts. (A) The first part was an epiluminescence detector (Figure 1), which consisted of microscope objective (magnification 25×, numerical aperture 0.40 or magnification 100×, numerical aperture 1.25, Olympus), dichroic mirror (900 nm short pass, ThorLabs), optical filters (875 nm short pass and 925 nm long pass, Edmund Optics) and a CCD array spectrometer (QE65000, Ocean Optics). In each scanned point, the emission spectrum of photon-upconversion was recorded in a range from 430 to 875 nm. The spectroscope was optionally replaced with an sCMOS camera for single nanoparticle imaging (Zyla 5.5, Andor). Photon-upconversion luminescence was excited by 976 nm 1 W laser diode (Laserland). The dichroic mirror reflected the excitation laser beam on a scanned object through a microscope objective. The same microscope objective also collected emitted luminescence into the spectroscope. (B) The second part of the scanner was a xy-moving stage, which holds the epiluminescence detector and allowed its moving in two dimensions with step of 200 μm. The moving stage was controlled by Arduino Nano board. A universal serial bus was used for communication with personal computer with the Windows 10 operating system. A graphical Java application was used for controlling the scanner xy-moving stage and recording data from the spectroscope. The output data were saved in the form of text files.

![Figure 1](image)

**Figure 1** The scheme of epiluminescence detector. The dichroic mirror reflects the excitation laser beam (976 nm) on a scanned object through a microscope objective (black line). The same microscope objective also collects emitted luminescence from UCNPs into the spectroscope (red line).

2.2. Nanoparticle synthesis and characterization

According to previous reports, the nanoparticles were prepared by thermal decomposition of oleates at high temperature (~300 °C) [20]. After surface coating by carboxylated silica, the nanoparticles created stable dispersions in water [20,21]. The mass concentration of UCNPs in water was measured by a gravimetric method: The volume of 200 μL of UCNPs dispersion was loaded into a glass vial. The vial was placed first on a heater to evaporate water and then for 3 hours in an oven at 450 °C and the mass of remaining UCNPs was measured. To estimate the hydrodynamic diameters of UCNPs, dynamic light scattering (DLS) of UCNP water or cyclohexane dispersions was measured in a glass cuvette with a 10 mm optical path. The volume of dispersion was 1000 μL and the mass concentration of UCNPs was 0.5 mg mL⁻¹.
3. RESULTS AND DISCUSSION

3.1. Photon-upconversion nanoparticles

Four types of UCNPs were prepared (Table 1). Three types of photon-upconversion nanoparticles (UCNP1-3) were prepared for hyperspectral scanning on a microtiter plate. The sizes of the luminescent core of these nanoparticles were approximately 30 nm. The chemical composition of these UCNPs differed in the type of activator ion (either Tm$^{3+}$, Er$^{3+}$ or Ho$^{3+}$). The fourth nanoparticle type (UCNP4) with 127 nm luminescent core was used for the measurement of single nanoparticle emission spectrum. The emission spectra of UCNP1-4 (Figure 2) were recorded from water dispersions (100 µL per well) on a microtiter plate by photon-upconversion laser scanner. The emission spectra revealed narrow emission peaks, which are typical for Ho$^{3+}$, Er$^{3+}$ and Tm$^{3+}$ doped UCNPs as it is shown in Figure 2.

Table 1 The list of prepared photon-upconversion nanoparticles

<table>
<thead>
<tr>
<th>Nanoparticle type</th>
<th>Nanoparticle composition</th>
<th>Hydrodynamic diameter without silica shell in cyclohexane (nm)</th>
<th>Hydrodynamic diameter with silica shell in water (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCNP1</td>
<td>NaY$<em>{0.80}$Yb$</em>{0.18}$Ho$_{0.02}$F$_4$</td>
<td>30.5</td>
<td>48.4</td>
</tr>
<tr>
<td>UCNP2</td>
<td>NaY$<em>{0.80}$Yb$</em>{0.18}$Er$_{0.02}$F$_4$</td>
<td>31.2</td>
<td>83.5</td>
</tr>
<tr>
<td>UCNP3</td>
<td>NaY$<em>{0.80}$Yb$</em>{0.18}$Tm$_{0.02}$F$_4$</td>
<td>28.4</td>
<td>39.4</td>
</tr>
<tr>
<td>UCNP4</td>
<td>NaY$<em>{0.80}$Yb$</em>{0.18}$Er$_{0.02}$F$_4$</td>
<td>127</td>
<td>158</td>
</tr>
</tbody>
</table>

Figure 2 Emission spectra of UCNPs. Emission spectra of UCNP water dispersions measured from the microtiter plate by a photon-upconversion laser scanner (100 µL per well, integration time 1 s). The concentrations of UCNPs were 10 mg mL$^{-1}$, 0.25 mg mL$^{-1}$, 0.13 mg mL$^{-1}$ and 0.25 mg mL$^{-1}$ for UCNP1-4, respectively.

3.2. Scanning of nanoparticle dispersions on a microtiter plate

Dispersions containing either UCNP1, UCNP2 or UCNP3 were prepared and dispensed into 96-well clear microtiter plate (100 µL per well). Utilizing a microscope objective with magnification 25× and numerical aperture 0.4, the plate was scanned with a spatial resolution of 200 µm. The integration time of 10 ms was used for recording the emission spectrum from each point with a reading speed of 57 points per second. To show the advantages of hyperspectral scanning, we recorded the sum of emission intensities for three different spectral ranges, i.e., detection channels. Those channels were subsequently used for constructing colored image (red channel: emission 621-707 nm, green channel: emission 512-572 nm, blue channel: emission 759-.
860 nm, **Figure 3**). From **Figure 3**, it is clear that hyperspectral scanning allows projecting different types of nanoparticles as different colors, which can be advantageously used for signal multiplexing.

**Figure 3** Hyperspectral scanning of UCNP dispersions on a microtiter plate. Dispersions of UCNP1-3 were loaded on 96 well microtiter plate (100 µL per well, 10 mg mL\(^{-1}\), 0.25 mg mL\(^{-1}\) and 0.13 mg mL\(^{-1}\) for UCNP1-3, respectively) and scanned with a resolution of 200 µm with 10 ms integration time per scanned point. Three channels are projected for three different spectral ranges (red channel: emission 621-707 nm, green channel: emission 512-572 nm, blue channel: emission 759-860 nm). These channels were projected as a colored image (red/green/blue).

### 3.3. Recording the emission spectrum of single photon-upconversion nanoparticle

We further tested whether the scanner is sensitive enough to record the emission of single nanoparticle on a planar surface, which can be important for the construction of photon-upconversion nanosensors. To record the emission spectrum of single nanoparticle, the glycerol immersion objective was used for focusing the excitation laser and collecting the emitted luminescence. The nanoparticles (UCNP4) were adsorbed on microscopic cover glass (150 µm thickness) and imaged by sCMOS camera (integration time 30 ms) as it was described previously. The emission spectrum was recorded (integration time 10 s, **Figure 4**) when the area of the substrate containing only single nanoparticle in the field of view was selected.

**Figure 4** Emission spectrum of single UCNP. The emission spectrum of a single UCNP4 was recorded. The inset shows the image of single UCNP4 from sCMOS camera.

### 4. CONCLUSION

We constructed a hyperspectral epiluminescence laser scanner, which allows recording an emission spectrum in each point of the scanned image. The scanner provides an advantage over laser scanners, which are able to record only the sum of intensities within single range of wavelengths. In this study we have shown that multiple detection channels can be extracted from recorded data and those channels can be used for
constructing colored images; different colors are projected for different types of photon-upconversion nanoparticles. We suppose that this multichannel sensing capability can be useful for analytical signal multiplexing and the development of multiplexed assays. We further showed that the scanner detector is sensitive enough even for recording the emission spectrum from single photon-upconversion nanoparticle, which can be useful for biosensor developing on the bases of single photon-upconversion nanoparticles.

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