9th International Conference on Nano-Molecular Electronics

Optimization of microscope unit for studying fluorescence emitters under high-vacuum and ambient gas conditions: Optical properties for various ionic liquids as a refractive index matching medium

Toshiki Yamada*, Akira Otomo
Kobe Advanced ICT Research Center, National Institute of Information and Communications Technology, 588-2 Iwaoka, Kobe 651-2492, Japan

Abstract

We briefly introduce our microscope unit with an immersion objective and ionic liquid used as a refractive index matching medium, and describe its application to single molecular spectroscopy under high-vacuum and various ambient gas conditions. In this article, we particularly focus our attention on studies toward optimizing the microscope unit. For this purpose, the refractive index and background fluorescence, absorption spectrum for various ionic liquids were investigated. We also measured the images of the beam spot of excitation laser at focal point by using an immersion objective and various ionic liquids. We found that some have more suitable characteristics than before.

© 2010 Published by Elsevier B.V. Open access under CC BY-NC-ND license.

Keywords: Time-correlated single photon counting system; immersion objective; ionic liquid; refractive index matching; high vacuum

1. Introduction

Single molecule spectroscopy along with use of the time-correlated single photon counting (TCSPC) system has been widely applied in studying the emission characteristics of individual organic fluorescent molecules [1], individual colloidal semiconductor nanocrystals [2], and individual bio-molecules labeled with fluorescent moieties [3]. The studies conducted on such single-photon emitters include measurements of fluorescence decay curves, emission spectra, fluorescence correlation spectroscopy, fluorescence intensity time traces for investigating photobleaching and blinking phenomena, and the characterization of single-photon sources (photon antibunching) by using the Hanbury-Brown and Twiss setup [4-6]. Avalanche photodiodes (APDs) of high quantum efficiency are commonly used as photon detectors [4-8]. Single molecular spectroscopy in conjunction with a sensitive CCD camera is also important. Orientation imaging of single molecules by wide-field illumination has been performed [9].

Previously, we developed a measurement system for single molecular spectroscopy, and demonstrated the usefulness of the TCSPC system with modified photomultiplier tubes (PMTs) as photon detectors, which has high

---

* Corresponding author. Tel.: +81-78-969-2257; Fax: +81-78-969-2259.
E-mail address: toshiki@nict.go.jp.
quantum efficiency, a relatively large active area, and extremely small dark counts [10]. Efficient collection of photons emitted from single emitters is also important for single molecular spectroscopy. Therefore, a general objective with a high numerical aperture (NA) (~0.95), or more preferably, an oil immersion objective with a high NA (1.3–1.4) is used. For some studies in single molecular spectroscopy, the measurements of the emission characteristic of single emitters under various ambient conditions, such as high vacuum and pure gas, are required. Objective lenses having a long working distance and placed in ambient air are conventionally used for photo-excitation of a sample and collection of photons emitted from the sample through the optical window that separates the high-vacuum atmosphere from the ambient air. However, the typical NA of an objective lens with a long working distance is not so high; about 0.6. Previously we proposed a new microscope unit that has an immersion objective and ionic liquid used as a refractive index-matching medium [11], and demonstrated the usefulness of the microscope unit for single molecular spectroscopy under various ambient conditions, including high vacuum and pure gas [12], though studies toward optimization of the microscope unit have not been performed.

Recently, ionic liquids [13, 14] have been extensively studied because of their unique properties, such as a wide liquid range, high electrical conductivity, extremely low vapor pressure, excellent thermal stability, and low combustibility. The characteristics of ionic liquids required for our purpose are non-volatility in a high vacuum, refractive index matching, and small background fluorescence. After briefly reviewing our previous works on the new microscope unit and the application to single molecular spectroscopy, in this article, we focus on studies toward the optimizing the unit. For this purpose, the refractive index, the absorption spectrum and background fluorescence for various ionic liquids were investigated. In addition, we also measured the images of the beam spot of excitation laser at focal point by using an immersion objective and various ionic liquids, which are related to the resolution in the present system. We found that some have more suitable characteristics than before. Thus our microscope unit is expected to have a wide range of applications for numerous optical measurements that require high resolution and brightness under high-vacuum, and various ambient gas conditions.

2. Experimental

Fig. 1(a) shows our experimental setup. Our laser and TCSPC systems were described elsewhere [10, 11]. A laser with a wavelength of 446 nm, pulse width of 2 ps, and repetition frequency of 8 MHz, as an excitation source, was used. The sample located in our microscope unit was excited by the laser beam. The fluorescence from the sample was detected with a modified photomultiplier tube (Hamamatsu H7422P-40-MOD). The data of each detected photon were recorded by TCSPC. Our microscope unit consists of a turbo molecular pump, gas purge line, small vacuum chamber with a synthesized quartz window, sample substrate, sample holder, XY- and Z-translation stages, an immersion objective with an NA of 1.3 and magnification of 100×, and an ionic liquid. A synthesized quartz cover slip substrate was used as the sample substrate. A droplet of the ionic liquid was placed on the immersion objective. The sample substrate was positioned on the droplet. The vacuum was maintained at about 8 × 10⁻⁶ Torr. This indicates that the ionic liquid is non-volatile under high-vacuum conditions. When the measurements were performed under ambient gas conditions, the vacuum chamber was purged with a pure gas with an appropriate pressure after evacuation. Thus the inside of our microscope unit offers ambient gas conditions at an appropriate pressure, as well as high-vacuum conditions.

The ionic liquid, N,N-diethyl-N-(2-methoxyethyl) ammonium bis(trifluoromethanesulfonyl)imide was used as a refractive index matching medium, in our previous experiments [10, 11]. The ionic liquid had a small background fluorescence appropriate for our experimental conditions. Using this ionic liquid, we confirmed the usefulness of the microscope units and applied it to single molecular spectroscopy for colloidal CdSe/ZnS core/shell nanocrystals on the synthesized quartz cover slip substrate under the ambient air, high-vacuum, and pure N₂ gas conditions. Emission characteristics, such as fluorescence decay curves, fluorescence intensity time trace, and photon antibunching were investigated under the various ambient conditions. In Figs. 1(b) and 1(c), some typical fluorescence decay curves for individual colloidal CdSe/ZnS core/shell nanocrystals under the ambient air and high-vacuum conditions are shown as examples. Similar data were also shown in [11].

Toward the optimization of our microscope unit, in this article, we investigated the optical characteristics of various ionic liquids, such as imidazolium-based, pyridinium-based, alkyl ammonium-based, alicyclic ammonium-based, and phosphonium-based, from the viewpoints of refractive index matching and background fluorescence.
The background fluorescence was measured by using an experimental setup as shown in Fig. 1(a). The image of the laser beam spot at focal point was also measured by a CCD in the reflection direction. The refractive index for various ionic liquids was measured by a conventional refractometer. The absorption spectrum for various ionic liquids was also measured by a spectrometer. In the measurement, the ionic liquid was filled in a quartz cell with an optical path of 1 mm.

Fig. 1. (a) Experimental setup. (b) and (c) Typical fluorescence decay curves for a single CdSe/ZnS core/shell nanocrystal (QD 565) in a PMMA matrix under ambient air and high-vacuum conditions, respectively. Data was recorded for 30 s with 50/4,096 ns time division. Excitation power was about 120 W/cm².

3. Results and Discussion

As most ionic liquids show extremely small vapor pressure and non-volatility in a high vacuum, the refractive index matching and small background fluorescence are essential for our purpose. In our previous works, we were concerned with the small background fluorescence, but less concerned about the refractive index matching. Yet the latter is an important factor for further optimization. Therefore we measured the refractive index of various ionic liquids. Counts due to background fluorescence for various ionic liquids were also measured. The experimental setup shown in Fig. 1(a) was used for the measurements. A pre-cleaned synthesized quartz cover slip was used as the sample substrate. For the comparison, the counts due to background fluorescence when using conventional non-fluorescence-type, Nikon NF immersion oil was also measured, while the microscope unit was not evacuated.

Fig. 2 summarizes the studied ionic liquids with a reference number and the name of companies from which they were purchased or provided. In Table 1, the reference number of the ionic liquid, refractive index, and background fluorescence counts are summarized.

We found that the well-known imidazolium-based ionic liquid (1) and pyridinium-based ionic liquid (2) showed strong background fluorescence that disturbs the single molecular spectroscopy. This may be due to its π-conjugation in these molecules. The alkyl or alicyclic ammonium-based ionic liquids (3-8) showed relatively small background fluorescence. Among these ammonium-based ionic liquids, those with tris(pentafluoroethyl)trifluorophosphate anion (7, 8) showed a slightly stronger background fluorescence. The refractive index of most of these ionic liquids is in the range of 1.40–1.45. The refractive index of N,N-diethyl-N-(2-methoxyethyl)ammonium bis(trifluoromethanesulfonyl)imide (3), which was used in our previous studies, was 1.42. As the refractive index of the synthesized quartz coverslip is about 1.48, there is a small mismatch in the refractive index.

Next we focused on phosphonium-based ionic liquids (9-15). We found that some have a relatively high refractive index of 1.45–1.50 and show relatively small background fluorescence. As the refractive index of the synthesized quartz coverslip is about 1.48 and that of general glass coverslip is about 1.51, these phosphonium-based ionic liquids are promising from the viewpoint of refractive index matching. Notably, trihexyltetradecylphosphonium
chloride (9) and IL-AP3-3 (15) have refractive indexes of 1.48 and 1.50, respectively, and show small background fluorescence. Thus, we found more appropriate ionic liquids from the viewpoints of refractive index matching and small background fluorescence. These ionic liquids are promising for single molecular spectroscopy under high-vacuum and various ambient gas conditions with the use of a synthesized quartz coverslip or general glass coverslip substrate.

Table 1. Reference numbers (R.N.) of the ionic liquid, refractive index (n), and background fluorescence (B.F.) counts are summarized. Excitation power density was about 150 W/cm² for the measurement of background fluorescence counts. It should be mentioned that the background fluorescence counts with use of Nikon NF immersion oil is 40–50 counts/s.

<table>
<thead>
<tr>
<th>R.N.</th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5.</th>
<th>6.</th>
<th>7.</th>
<th>8.</th>
<th>9.</th>
<th>10.</th>
<th>11.</th>
<th>12.</th>
<th>13.</th>
<th>14.</th>
<th>15.</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1.42</td>
<td>1.45</td>
<td>1.42</td>
<td>1.41</td>
<td>1.41</td>
<td>1.43</td>
<td>1.42</td>
<td>1.37</td>
<td>1.48</td>
<td>1.42</td>
<td>1.48</td>
<td>1.50</td>
<td>1.44</td>
<td>1.45</td>
<td>1.50</td>
</tr>
<tr>
<td>B.F.</td>
<td>&gt;10⁵</td>
<td>&gt;10³</td>
<td>~50</td>
<td>~60</td>
<td>~60</td>
<td>~120</td>
<td>~120</td>
<td>~50</td>
<td>&gt;10⁵</td>
<td>&gt;10³</td>
<td>&gt;10³</td>
<td>~50</td>
<td>~50</td>
<td>~50</td>
<td>~50</td>
</tr>
</tbody>
</table>

Furthermore, we measured the images of the beam spot of excitation laser at focal point by using the immersion objective and some ionic liquids as a refractive index matching medium. Fig. 3(a) shows the images of the beam spot by using N,N-diethyl-N-(2-methoxyethyl)ammonium bis(trifluoromethanesulfonyl)imide (3), which was used in our previous studies. Figs. 3(b), 3(c) and 3(d) show the images of the beam spot by using trihexyltetradecylphosphonium chloride (9), IL-AP3-3 (15) and Nikon NF immersion oil, respectively. When using Nikon immersion NF, we measured in ambient air. The sizes of the beam spot by using the ionic liquids; (3), (9), (15) and Nikon NF immersion oil were about 1.1, 0.7, 0.6 and 0.6 μm, respectively. Thus, we found the improvement by using the ionic liquids; (9) and (15), due to the better refractive index matching, compared with the ionic liquid (3) used in our previous studies.

The absorption spectra are also measured for various ionic liquids. The absorption spectra for the ionic liquids; (3), (9), (15) and Nikon NF immersion oil are shown in Fig. 4. There are no absorption bands in visible region for...
these samples. N,N-diethyl-N-(2-methoxyethyl)ammonium bis(trifluoromethanesulfonyl)imide (3) and IL-AP3-3 (15) are more transparent in ultra violet region, compared with Nikon NF immersion oil. Especially, ionic liquid (3) is highly transparent in ultra violet region. Apart from the present work, the characteristics is very useful when we use the ionic liquids as a refractive index matching medium in ultra violet region under high-vacuum, and various ambient gas conditions.

![Absorption spectra for the ionic liquids: (3), (9), (15) and Nikon NF immersion oil](image)

**Fig. 4. Absorption spectra for the ionic liquids: (3), (9), (15) and Nikon NF immersion oil**

### 4. Conclusion

In this paper, we briefly described our microscope unit and its application to single molecular spectroscopy. We particularly focused our attention on studies toward optimization of the unit. For this purpose, we investigated the optical properties of various ionic liquids and found a greater number of appropriate ones than before. Our microscope unit is expected to have a wide range of applications for a range of optical measurements, such as Raman spectroscopy and nonlinear optical spectroscopy, which require high resolution and brightness under high-vacuum and various ambient gas conditions.

### Acknowledgements

Part of this work was supported by a Grant-in-Aid for Scientific Research (C) (20510112).

### References