An organic dye with very large Stokes-shift and broad tunability of fluorescence: Potential two-photon probe for bioimaging and ultra-sensitive solid-state gas sensor

He, Tingchao; Wang, Yue; Tian, Xiaoqing; Gao, Yang; Zhao, Xin; Grimsdale, Andrew Clive; Lin, Xiaodong; Sun, Handong

2016

He, T., Wang, Y., Tian, X., Gao, Y., Zhao, X., Grimsdale, A. C., et al. (2016). An organic dye with very large Stokes-shift and broad tunability of fluorescence: Potential two-photon probe for bioimaging and ultra-sensitive solid-state gas sensor. *Applied Physics Letters*, 108(1), 011901-.

<https://hdl.handle.net/10356/82387>

<https://doi.org/10.1063/1.4939232>

© 2016 American Institute of Physics. This paper was published in *Applied Physics Letters* and is made available as an electronic reprint (preprint) with permission of American Institute of Physics. The published version is available at: [<http://dx.doi.org/10.1063/1.4939232>]. One print or electronic copy may be made for personal use only. Systematic or multiple reproduction, distribution to multiple locations via electronic or other means, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper is prohibited and is subject to penalties under law.

**An organic dye with very large Stokes-shift and broad tunability of fluorescence:
Potential two-photon probe for bioimaging and ultra-sensitive solid-state gas sensor**

Tingchao He, Yue Wang, Xiaoqing Tian, Yang Gao, Xin Zhao, Andrew C. Grimsdale, Xiaodong Lin, and Handong Sun

Citation: *Applied Physics Letters* **108**, 011901 (2016); doi: 10.1063/1.4939232

View online: <http://dx.doi.org/10.1063/1.4939232>

View Table of Contents: <http://scitation.aip.org/content/aip/journal/apl/108/1?ver=pdfcov>

Published by the AIP Publishing

Articles you may be interested in

[Polarization induced control of single and two-photon fluorescence](#)

J. Chem. Phys. **132**, 154508 (2010); 10.1063/1.3386574

[Improvement in the reduction of silver ions in aqueous solution using two-photon sensitive dye](#)

Appl. Phys. Lett. **89**, 113102 (2006); 10.1063/1.2345601

[Crossed-beam two-photon readout system for three-dimensional radiation dosimeters](#)

Rev. Sci. Instrum. **73**, 4214 (2002); 10.1063/1.1518789

[Two-photon photopolymerization and diagnosis of three-dimensional microstructures containing fluorescent dyes](#)

Appl. Phys. Lett. **79**, 1411 (2001); 10.1063/1.1399312

[Absorption bleaching of squarylium dye J aggregates via a two-photon excitation process](#)

Appl. Phys. Lett. **79**, 708 (2001); 10.1063/1.1390322

The advertisement features a blue background with a molecular structure of spheres and sticks. On the left is a thumbnail image of the 'AIP Applied Physics Reviews' journal cover, which shows a diagram of a device structure. The main text 'NEW Special Topic Sections' is in large white font. Below it, 'NOW ONLINE' is in yellow, followed by 'Lithium Niobate Properties and Applications: Reviews of Emerging Trends' in white. The AIP Applied Physics Reviews logo is in the bottom right corner.

NEW Special Topic Sections

NOW ONLINE
Lithium Niobate Properties and Applications:
Reviews of Emerging Trends

AIP Applied Physics Reviews

An organic dye with very large Stokes-shift and broad tunability of fluorescence: Potential two-photon probe for bioimaging and ultra-sensitive solid-state gas sensor

Tingchao He,¹ Yue Wang,² Xiaoqing Tian,¹ Yang Gao,³ Xin Zhao,² Andrew C. Grimsdale,³ Xiaodong Lin,^{1,a)} and Handong Sun^{2,a)}

¹College of Physics Science and Technology, Shenzhen University, Shenzhen 518060, China

²Division of Physics and Applied Physics, and Centre for Disruptive Photonic Technologies (CDPT), School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371, Singapore

³School of Materials Science and Engineering, Nanyang Technological University, Singapore 639798, Singapore

(Received 26 August 2015; accepted 18 December 2015; published online 4 January 2016)

Light-emitting nonlinear optical molecules, especially those with large Stokes shifts and broad tunability of their emission wavelength, have attracted considerable attention for various applications including biomedical imaging and fluorescent sensors. However, most fluorescent chromophores have only limited potential for such applications due to small Stokes shifts, narrow tunability of fluorescence emissions, and small optical nonlinearity in highly polar solvents. In this work, we demonstrate that a two-photon absorbing stilbene chromophore exhibits a large two-photon absorption action cross-section ($\eta\delta = 320 \text{ GM}$) in dimethylsulfoxide (DMSO) and shows broad fluorescence tunability (125 nm) by manipulating the polarity of the surrounding medium. Importantly, a very large Stokes shift of up to 227 nm is achieved in DMSO. Thanks to these features, this chromophore can be utilized as a two-photon probe for bioimaging applications and in an ultrasensitive solid-state gas detector. © 2016 AIP Publishing LLC.

<http://dx.doi.org/10.1063/1.4939232>

Fluorescent compounds, especially those with tunable wavelengths, have been extensively studied for several decades, and much progress has been made for device applications, including light-emitting diodes, sensing technology, bioimaging agents, and key components of other optoelectronic devices.^{1–4} Among various applications, sensing technology has made a significant progress by taking advantage of a large number of fluorescent materials that provide a particularly useful platform, which have shown great advantages, especially in sensitivity, low cost, and simplicity.^{5–12}

Since fluorescent sensors rely on the use of intrinsic fluorophores, the development of fluorophores remains an essential element of the design of fluorescent sensors. Unfortunately, there are two factors that limit the full potential of fluorescent sensors. On the one hand, most of these fluorescent probes are excited by a one-photon method using a short wavelength, which results in some obvious drawbacks, such as high autofluorescence background, photo-bleaching, and shallow penetration depth.^{13–15} On the other hand, it is known that organic molecules with large Stokes shifts (particularly those with Stokes shifts $>80 \text{ nm}$) are very promising, since in that case low background interference in bioimaging can be achieved.^{16,17} However, most known fluorescent molecules exhibit small Stokes shifts. Meanwhile, the narrow tunability of fluorescence emissions and small optical nonlinearity in highly polar solvents also make the realization of their full potential a daunting task. As a consequence, the combination of a large Stokes-shift and efficient

two-photon absorption (TPA) within organic dyes in highly polar solvents would be especially useful for fully developing versatile fluorescent sensors. Herein, we use the two-photon approach to develop an efficient fluorescent probe for bioimaging applications and chloroform (CHCl_3) vapor detection. The probe molecule exhibits a large TPA action cross-section ($\eta\delta = 320 \text{ GM}$) and a very large Stokes shift (227 nm) in dimethylsulfoxide (DMSO), as well as broad tunability of fluorescence emission (125 nm) across different media. Benefiting from the abovementioned advantages, the probe not only can be used for two-photon fluorescence imaging in live cells but also shows a pronounced fluorescent response toward CHCl_3 vapor.

The TPA and third-harmonic generation properties of the stilbene chromophore (*E*)-[4-(6-bromohexyloxy)phenyl]-2-(4-nitrophenyl)ethene have been investigated in our previous work.¹⁸ However, the influence of the surrounding media on its fluorescence and nonlinear optical properties had not been investigated, which may be useful for exploring possible other applications. In order to investigate the influence of the medium's permittivity on the electronic absorption and emission spectra of the stilbene chromophore, toluene, CHCl_3 , and DMSO were chosen as solvents owing to the large difference in their polarities. Meanwhile, the chromophore was also doped into a poly(methylmethacrylate) (PMMA) film with the aim of exploring its potential applications in solid optoelectronic devices. Figure 1 depicts the normalized electronic absorption and emission spectra of stilbene chromophore in above media at room temperature. It was found that the absorption spectrum was relatively insensitive to the changes in the polarity of the environment,

^{a)} Authors to whom correspondence should be addressed. Electronic addresses: linxd@szu.edu.cn and hdsun@ntu.edu.sg

FIG. 1. Normalized optical absorption and fluorescence spectra of the stilbene chromophore in different media. The insets show photographs of fluorescence emission for the stilbene chromophore in toluene (1), CHCl_3 (2), and DMSO (3) under UV excitation.

with only a small shift in different media. By contrast, the maximum emission wavelength was red-shifted by 125 nm from 490 nm in toluene to 615 nm in DMSO. This suggested that a strong intramolecular charge transfer (ICT) phenomenon occurred.¹⁹ In this case, upon electronic transition to the ICT excited state, a large change in the dipole moment would be induced in the donor- π -acceptor stilbene molecule, which led to a large sensitivity of emission wavelength on the polarity of solvent. At the same time, the fluorescence quantum yield (QY) increased significantly from toluene (<0.1%) to DMSO (30%) due to reduced nonradiative recombination (supplementary material²⁹). Notably, the chromophore in DMSO exhibits a very large Stokes shift of 227 nm, which represents one of the largest values among fluorescent materials, and is strongly desirable in candidates for use in fluorescent sensors.¹⁹ Additionally, the high sensitivity of the fluorescence wavelength of stilbene chromophore to the surrounding media of different polarities gives it high potential as a sensing material, which shows good prospects for use in various applications. Considering the non-toxicity of DMSO towards cell culture and the high QY of the molecule in DMSO, this chromophore should have a strong potential in biological imaging. Regarding the fluorescence spectrum in the PMMA film, due to the molecular packing induced crystallographic modification, it exhibited a large blue shift of fluorescence emission ($\lambda_{\text{em}} = 515$ nm) compared to the case in CHCl_3 solution ($\lambda_{\text{em}} = 565$ nm). Inspired by this unusual phenomenon, the PMMA film of chromophore should be promising as a CHCl_3 vapor sensor.

The nature of the transitions at the molecular level can be easily understood based on quantum chemical calculations.²⁰ The molecular orbital calculation results suggested that the highest occupied molecular orbital (HOMO) was concentrated at the styrene center while the lowest unoccupied molecular orbital (LUMO) electron density was located mainly on the nitro-group of the stilbene chromophore (Fig. 2). The separate distribution of the localized nature of energy levels will induce strong ICT properties and large TPA cross-section.²⁰ It would make it be very useful in two-photon probes for bioimaging and ultra-sensitive solid-state gas sensors.

FIG. 2. Frontier HOMO/LUMO energy of stilbene chromophore calculated with DFT at the B3LYP/6-31G(d) level by applying the Gaussian 09 program. The HOMO and LUMO distributions are linked by the styrene core. Consequently, long conjugation is formed when nitro-group is added onto the structure.

From Fig. 1, one can also see that there is one spectral window for the chromophore solution with negligible one-photon absorption above 600 nm, which is suitable for two-photon excitation at the wavelength from 720 nm to 920 nm. To verify that the observed fluorescence was the result of TPA, we measured the emission intensity as a function of the input excitation intensity at 800 nm (Fig. 3(a)). The slopes of the best fitting straight lines were 1.97, 2.01, and 1.97 for the chromophore in DMSO, CHCl_3 and toluene, respectively, which confirmed the TPA mechanism for 800 nm excitation.²¹ The dynamic relaxation processes of the stilbene chromophore under two-photon excitation were studied, with the help of a high-speed streak camera system from Optronis, to provide insight into the emission

FIG. 3. (a) Quadratic dependence of TPA induced fluorescence on the excitation intensity in different organic solvents. (b) Fluorescence decay of two-photon excited fluorescence in different organic solvents.

FIG. 4. TPA spectra of stilbene chromophore in toluene, CHCl₃ and DMSO.

mechanism and determine the best strategies for related applications. As illustrated in Fig. 3(b), the fluorescence lifetimes of the stilbene chromophore in CHCl₃ and DMSO were much longer than in toluene as a result of a drastic increase in the rate of nonradiative decay. This was also responsible for the very low fluorescence QY in toluene.²²

The TPA cross-sections of stilbene chromophore in toluene, CHCl₃, and DMSO have been measured in the 720–920 nm range by means of the two-photon excited fluorescence method,²¹ as depicted in Fig. 4. In the measurement, a low pass filter (<700 nm) was used to block the scattering of excitation light. The corresponding maximum 2PA cross-sections were 4.6 GM, 470 GM, 1068 GM, respectively. Most of the reports on TPA fluorescent probes have focused only on either the enhancement of the fluorescence QY (η) or the TPA cross-section (δ). However, one must maximize the product of η and δ to guarantee a sensitive response in sensing and imaging.²³ The TPA action cross-section of the stilbene chromophore in DMSO was calculated to be 320 GM. This value was among the largest TPA action cross-sections of organic molecules in highly polar solvents. Meanwhile, the dye maintained a high TPA cross-section across the range of 760–820 nm, which followed into output wavelengths of the most available femtosecond excitation source, offering much greater flexibility in the selection of excitation wavelength.

Encouraged by the high TPA action cross-section of stilbene chromophore in DMSO, we applied stilbene chromophore as a two-photon biological probe, which was investigated using a microscope (product model: Olympus BX41) under 800 nm excitation. 3-(4,5-Dimethylthiazol-2-

yl)-2,5-diphenyltetrazolium bromide (MTT) assay revealed that the cellular viability of HeLa cells was greater than 90% after incubation with 200 μ M probe for 24 h, indicating the low cytotoxicity of molecular probe (data not shown here). HeLa cells were first treated with stilbene chromophore nanoparticles (10 μ M, 4 h), followed by washing three times with phosphate-buffered saline (PBS, 10 μ M, pH 7.12). The HeLa cells treated with stilbene chromophore exhibited strong and stable intracellular red fluorescence (Fig. 5(b)). The overlay image (Fig. 5(c)), whose brightness was deliberately adjusted in order to obtain high contrast, further confirmed the localization of stilbene chromophore in the cytoplasm, indicating that stilbene chromophore probes can penetrate the cell membrane and be used for *in vivo* imaging. Our demonstration of bioimaging indicated that stilbene chromophore in DMSO would be a more excellent two-photon bioimaging probe in *in vivo* bioimaging, compared to most of previous molecules including stilbene chromophore in THF.¹⁸

Chloroform is widely used in industry and in medicine, but it is also hazardous. Consequently, a wide variety of technologies for the detection of CHCl₃ have been developed, based on either electronic or optical principles.²⁴ However, most of these have limitations associated with complicated fabrication steps or expensive detection equipment. For example, for conventional electrical response based gas sensor, much complicated fabrication process of optoelectronic devices was usually needed, although such kinds of sensors could also exhibit high sensitivity for gas detection.²⁵ From the experimental results presented above, the stilbene was moderately fluorescent in the solid state and exhibited a high fluorescence QY in CHCl₃, accompanied by the large spectral shift between these different phases. It is therefore plausible that the stilbene/PMMA film might be useful for sensing CHCl₃ vapor. Meanwhile, solid state gas sensors are the best candidates for the development of commercial gas sensors due to their numerous advantages, such as small sizes, high sensitivities, and possibility of online operation.²⁶ In our tests, instead of one-photon excitation, femtosecond pulses at 800 nm (two-photon excitation) were used as the excitation source due to the various advantages of the latter type of excitation over the former. For example, two-photon excitation can more easily penetrate complicated surroundings over the detected object. Additionally, due to the efficient two-photon fluorescence of stilbene chromophore in DMSO, a stilbene-based two-photon fluorescent sensor can also be used for the detection of harmful matter in living cells. Recent advances in laser technique have allowed

FIG. 5. Fluorescent images collected with two-photon fluorescence microscopy. The excitation wavelength was 800 nm and images were collected at 540–720 nm. (a) The bright field image of live HeLa cells stained with 1×10^{-5} M probe for 4 h. (b) The corresponding dark field image of HeLa cells. (c) The overlay image of (a) and (b).

miniaturization and lowered production costs of femtosecond pulse lasers. Therefore, two-photon probes for gas detection have become practical. To demonstrate the applicability of the stilbene/PMMA film for two-photon gas detection, its fluorescence spectra were monitored, before and after exposure to the equilibrated vapor of CHCl_3 for varied periods of time (60, 120, 180, and 220 s). A schematic diagram of the optical measurement setup is drawn in Fig. 6(a). The details on the preparation of the sample and detailed measurement process for CHCl_3 sensing are described in supplementary material.²⁹ As shown in Fig. 6(b), PMMA film of stilbene exhibited almost identically rapid and evident responses to the vapor under all conditions tested. It can be seen that fluorescence peak of PMMA film exhibited a gradual red shift with increased exposure time to CHCl_3 vapor. This observation can be well explained by the partial dissolution of chromophore microcrystals to produce solvated independent molecules with time. The CHCl_3 vapor was continuously diffusing into the vial where the PMMA film was fixed, which increased the local CHCl_3 concentration around the stilbene

FIG. 6. (a) Schematic diagram of the optical measurement setup. (b) Fluorescence spectra (collected with two-photon excitation) from the organic film versus diffusion time (corresponding to the concentration of CHCl_3 vapor). (c) The dependence of fluorescence intensity on the CHCl_3 gas concentration. (d) Dynamic shift of fluorescence emission wavelength versus the concentration of CHCl_3 vapor. The inset schematically illuminates fluorescence spectra at different time: “Before”—before the sensing vapor is added (red), “After”—after the vapor is added (green), and “Final”—the recovery of fluorescence emission from pristine film by simply heating the PMMA film at 120 °C for about 1 min.

molecules and changed the local dielectric constant of the medium. The red shift was up to 22 nm when the diffusion time was 220 s (corresponding to a vapor concentration of 660 ppm in air). Meanwhile, a large enhancement of fluorescence intensity (13 times) could also be observed (Fig. 6(c)). Such a large spectral shift and change of fluorescence intensity could be easily detected by cheap and compact spectrophotometers. In addition, the pristine film could be recovered by simply heating the PMMA film at 120 °C for about 1 min. It was found that the spectrum peaks were exactly the same before the vapor addition and after the recovery step, as illustrated in the inset of Fig. 6(d). We therefore concluded that the red shift of spectrum peak and fluorescence intensity enhancement was related to the increased number of CHCl_3 molecules surrounding the chromophore molecules. The dynamic change in the spectra is plotted in Fig. 6(d). It can be seen that the spectral shift value and fluorescence enhancement ratio were almost linearly proportional to the diffusion time. The limit of detection for stilbene-based materials, assuming a resolution of 3 nm for a common compact spectrometer, was 60 ppm (gas phase). This value was in the same order of magnitude as for state-of-art CHCl_3 vapor sensors based on a copper/polyaniline nanocomposite²⁷ and polyaniline doped with carboxylic acid functionalized multi-walled carbon nanotubes.²⁸ In addition, if the calculations were carried out based on the change of fluorescence intensity, the detection limit was significantly lower. The excellent performance of this sensor was ascribed to the high sensitivity of molecular fluorescence emission wavelength to the permittivity of surrounding medium and the high contact area of the PMMA film.

In summary, our experimental results indicate that the stilbene chromophore exhibits good nonlinearity excited brightness in DMSO. Its performance as a label for two-photon *in vitro* imaging has been tested in HeLa cells with encouraging results. Meanwhile, we have developed a simple, cheap but highly sensitive potential platform for CHCl_3 gas detection. It is believed that stilbene chromophore will provide great potential in applications for two-photon *in vivo* imaging and ratiometric fluorescent gas sensors.

We thank the Natural Science Foundation of China (NSFC Grant Nos. 11404219 and 11304206), Natural Science Foundation of Guangdong Province (Grant No. 2014A030313552), Natural Science Foundation of SZU (Grant No. 2014016) and State Key Laboratory of Advanced Optical Communication Systems and Networks, Shanghai Jiao Tong University, China (Grant No. 2015GZKF03007), and Strategic emerging industry development Special Fund of Shenzhen (JCYJ20150324141711613 and JCYJ20140418095735562). This research was also supported by the Singapore National Research Foundation through the Competitive Research Programme (CRP) under Project No. NRFCRP5-2009-04.

¹S.-N. Zhao, L.-J. Li, X.-Z. Song, M. Zhu, Z.-M. Hao, X. Meng, L.-L. Wu, J. Feng, S.-Y. Song, C. Wang, and H.-J. Zhang, *Adv. Funct. Mater.* **25**, 1463 (2015).

²H. Chen, W. Lin, W. Jiang, B. Dong, H. Cui, and Y. Tang, *Chem. Commun.* **51**, 6968 (2015).

³X. Yan, T. R. Cook, P. Wang, F. Huang, and P. J. Stang, *Nat. Chem.* **7**, 342 (2015).

- ⁴S. Hu, A. Trinchì, P. Atkin, and I. Cole, *Angew. Chem. Int. Ed.* **54**, 2970 (2015).
- ⁵K. Wen, S. Yu, Z. Huang, L. Chen, M. Xiao, X. Yu, and L. Pu, *J. Am. Chem. Soc.* **137**, 4517 (2015).
- ⁶Y. Ding, Y. Tang, W. Zhua, and Y. Xie, *Chem. Soc. Rev.* **44**, 1101 (2015).
- ⁷J. Peng, W. Xu, C. L. Teoh, S. Han, B. Kim, A. Samanta, J. C. Er, L. Wang, L. Yuan, X. Liu, and Y. Chang, *J. Am. Chem. Soc.* **137**, 2336 (2015).
- ⁸S.-L. Liu, R. Sheng, M. J. O'Connor, Y. Cui, Y. Yoon, S. Kurilova, D. Lee, and W. Cho, *Angew. Chem. Int. Ed.* **53**, 14387 (2014).
- ⁹Z. Wang, J. Wang, J. Li, M. Feng, G. Zou, and X. Huang, *Chem. Commun.* **51**, 3094 (2015).
- ¹⁰A. Ozawa, A. Shimizu, R. Nishiyabua, and Y. Kubo, *Chem. Commun.* **51**, 118 (2015).
- ¹¹X. Zhang, C. Wang, Z. Han, and Y. Xiao, *ACS Appl. Mater. Interfaces* **6**, 21669 (2014).
- ¹²A. Liua, M. Honga, W. Yang, S. Lu, and D. Xua, *Tetrahedron* **70**, 6974 (2014).
- ¹³A. K. Mandal, T. He, S. K. Maji, H. Sun, and Y. Zhao, *Chem. Commun.* **50**, 14378 (2014).
- ¹⁴H. D. Ha, M. Jang, F. Liu, Y. Cho, and T. S. Seo, *Carbon* **81**, 367 (2015).
- ¹⁵L. Guo and M. S. Wong, *Adv. Mater.* **26**, 5400 (2014).
- ¹⁶A. Y. Bochkov, I. O. Akchurin, O. A. Dyachenkob, and V. F. Travena, *Chem. Commun.* **49**, 11653 (2013).
- ¹⁷T. Guo, L. Cui, J. Shen, R. Wang, W. Zhu, Y. Xu, and X. Qian, *Chem. Commun.* **49**, 1862 (2013).
- ¹⁸T. He, S. Sreejith, Y. Gao, A. C. Grimsdale, Y. Zhao, X. Lin, and H. Sun, *Appl. Phys. Lett.* **106**, 111904 (2015).
- ¹⁹C. Huang, X. Peng, D. Yi, J. Qu, and H. Niu, *Sens. Actuators, B* **182**, 521 (2013).
- ²⁰O. O. Adegoke, I. H. Jung, M. Orr, L. Yu, and T. Goodson III, *J. Am. Chem. Soc.* **137**, 5759 (2015).
- ²¹G. S. He, L. Tan, Q. Zheng, and P. N. Prasad, *Chem. Rev.* **108**, 1245 (2008).
- ²²T. He, P. C. Too, R. Chen, S. Chiba, and H. Sun, *Chem. Asian J.* **7**, 2090 (2012).
- ²³P. Liu, S. Li, Y. Jin, L. Qian, N. Gao, S. Q. Yao, F. Huang, Q. Xu, and Y. Cao, *ACS Appl. Mater. Interfaces* **7**, 6754 (2015).
- ²⁴S. Virji, J. Huang, R. B. Kaner, and B. H. Weiller, *Nano Lett.* **4**, 491 (2004).
- ²⁵N. Y. Chan, M. Zhao, J. Huang, K. Au, M. H. Wong, H. M. Yao, W. Lu, Y. Chen, C. W. Ong, H. L. W. Chan, and J. Dai, *Adv. Mater.* **26**, 5962 (2014).
- ²⁶G. Korotcenkov, *Mater. Sci. Eng., B* **139**, 1 (2007).
- ²⁷S. Sharmaa, C. Nirkheaa, S. Pethkarb, and A. A. Athawalea, *Sens. Actuators, B* **85**, 131 (2002).
- ²⁸P. Kar and A. Choudhury, *Sens. Actuators, B* **183**, 25 (2013).
- ²⁹See supplementary material at <http://dx.doi.org/10.1063/1.4939232> for the measurements of fluorescence quantum yields, the details on the preparation of the sample, and the detailed measurement process for CHCl₃ sensing.