SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
OPTICAL SENSORS
PHOTOCHEMISTRY

Received
21 March 2014

Accepted
28 April 2014

Published
30 May 2014

Correspondence and requests for materials should be addressed to F.L. (longf04@ruc.edu.cn)

Simple and compact optode for real-time in-situ temperature detection in very small samples

Feng Long[1] & Hanchang Shi[2]

[1]School of Environment and Natural Resources, Renmin University of China, Beijing, 100872, China, [2]State Key Joint Laboratory of ESPC, School of Environment, Tsinghua University, Beijing, 100084, China.

Real-time in-situ temperature detection is essential in many applications. In this paper, a simple and robust optode, which uses Ruthenium (II) complex as a temperature indicator, has been developed for rapid and sensitive temperature detection in small volume samples (<5 μL). Transmission of excitation light and collection and transmission of fluorescence are performed by a homemade single-multi mode fiber coupler, which provides the entire system with a simple and robust structure. The photoluminescence intensity of Ruthenium (II) complex diminishes monotonically from 0°C to 80°C, and the response to temperature is rapid and completely reversible. When temperature is less than (or higher than) 50°C, a linear correlation exists between the fluorescence intensity and the temperature. Excellent agreement was also observed between the continuous and in situ measurements obtained by the presented optode and the discrete temperature values measured by a conventional thermometer. The proposed optode has high sensitivity, high photostability and chemical stability, a wide detection range, and thermal reversibility, and can be applied to real-time in-situ temperature detection of a very small volume biological, environmental, and chemical sample.

Temperature, a fundamental thermodynamic property of matter, plays critical roles in various biological and industrial processes that range from cellular activities (e.g. protein folding, DNA hybridization) and biotechnological procedures (e.g., polymerase chain reaction and thermotherapy) to chemical process and reaction monitoring[1–5]. The importance of temperature in such processes has fueled interest in the development of real-time in-situ temperature sensors. Traditional thermometers (e.g., liquid mercury or kerosene-filled temperature sensors) and recent developing thermocouples and infrared thermometers are hard to miniaturize and are unsuitable for temperature sensing in small volume samples (μL or less)[6]. Photoluminescence (PL) thermometry, which exploits temperature dependent changes in luminescence properties (e.g., excited state lifetime or emission intensity) of an indicator or probe, is a versatile optical technique for measuring local temperature[1–5]. This type of optical measurement is attractive because of its simplicity, excellent spatial and temporal resolution, immunity to high electromagnetic fields, and capability for long distance measurements[7,8]. Currently, three PL mechanisms, namely, steady-state fluorescence intensity, luminescence decay time, and ratiometric intensity, were in general used to measure temperature[1,9–11]. The PL decay time approach measures temperature by using the lifetime of temperature-dependent fluorophore. However, lifetime detection requires expensive and fast optoelectronic instruments and faces challenges in performing real-time in-situ temperature detection in a real sample[1,10]. Ratiometric intensity technique utilizes the intensity ratio of two emission bands of two fluorophores to represent the temperature assuming that they have the similar photobleaching effect[12]. However, this technique requires two fluorophores to be excited by different light resources, which complicates the detection system structure. Measuring steady-state intensity is the simplest approach. However, due to luminescent complexes in conventional optodes that often directly contact with the sample, the release of luminescent complexes may contaminate the detected samples or the matrix of samples may affect luminous efficiency, thereby leading to the incorrect results.

To date, a variety of luminescent complexes have been employed for temperature sensing, including ruthenium complex, quantum dots, upconverting nanoparticles, lanthanide complex, and conjugated polymer[7–10,13,14]. Ruthenium (II) complexes are attractive and frequently used for optical sensing of chemical parameters such as temperature, oxygen, and pH. These complexes have outstanding features such as good photoluminescence quantum yields, relative ease of synthesis and purification, electronic stability enhanced by symmetrical t^6_{2g}

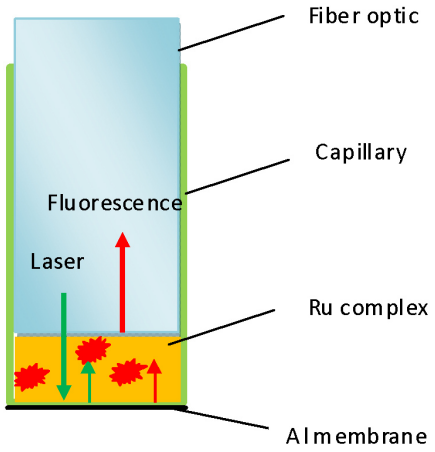

Figure 1 | **Schematic of ORMOSILs-based optode.**

configuration, moderately long excited state lifetimes, and chemical stability[13–16]. The PL intensity of Ruthenium (II) complex shows a strong dependence on the temperature, and the intensity variation provides a convenient means for detecting temperature changes[13,14]. Furthermore, Ruthenium (II) complex [e.g., $Ru(bpy)_3$ and Ru(phen)] is commercially available and can be easily incorporated into solid matrices by selecting an appropriate counterion. The use of sol-gel oxides (xerogels) is one of the most used fluorophore immobilization methods[15,16]. However, these inorganic sol-gel-based sensors commonly suffer from unstable response and nonlinear Stern–Volmer plots together with shrinkage. For a fluorescence intensity-based temperature sensor, variations could not only be caused by temperature variations but also by fluctuations in the local concentration of emitting centers. The use of Ruthenium (II) complex in optical temperature sensing generally requires a sophisticated encapsulation technique because its luminescence is known to be quenched by oxygen[15,16]. In addition, interferences by other oxidative or reductive quenchers (e.g., NO_x and SO_2) can be expected. More importantly, these sensors lack the ability for real-time in-situ quantitative detection, which is essential for monitoring in the study of microfluidics, micro-reaction, heat-driven hydrodynamics, and biological processes. Thus, challenges remain in achieving real-time in-situ quantitative temperature detection in small volume samples without affecting sample performances.

We fabricated a simply manufactured and highly miniaturized ruthenium complex-based optode for rapid and real-time in-situ temperature detection in a microenvironment. To improve sol-gel performance, organically modified silicates (ORMOSILs) were used because they allow the preparation of tailor-made xerogel-based composites to possess typical properties of organic materials, such as flexibility and mechanical strength, as well as benefits of inorganic materials, such as chemical resistance and high tensile strength[17]. To avoid the matrix effect of samples, ORMOSILs-based ruthenium complex was sealed in a capillary and was not in direct contact with samples. An all-fiber structure-based PL measurement system was used to detect temperature-dependent and time-resolved PL signals acquired from the ruthenium complex, which is easy-to-fabricate, highly compact, and has excellent light collection efficiency.

Results

Preparation and characteristics of the ORMOSILs-coated optode. The performance of temperature optode strongly depends on the immobilization method used to host the temperature-sensitive luminophore[18]. Figure 1 shows that an optode using Ruthenium (II) complex as a temperature indicator was prepared for in-situ temperature sensing. The distal end of the fiber optic probe was coated a ORMOSILs-based film containing Ruthenium (II) complex. The probe was inserted into a capillary, the end of which was sealed by a glass sheet deposited aluminum film to enhance the excited efficiency of the Ruthenium complex due to the reflected laser. At the same time, part of the excited fluorescence is reflected into the fiber optic probe, thereby increasing the detected fluorescence value.

Figure 2 shows Fourier transform infrared spectroscopy (FTIR) spectra of the fiber optic probe and the ORMOSILs-coated probe[19], respectively. For the fiber optic probe, only strong peaks at 1,070 and 795 cm^{-1} were shown; these peaks correspond to asymmetric and symmetric stretching vibration of Si-O-Si bond, respectively. However, the ORMOSILs-coated probe showed several strong peaks due to existing 3,3,3-trifluoropropyltrimethoxysilicane (TFP-TriMOS)

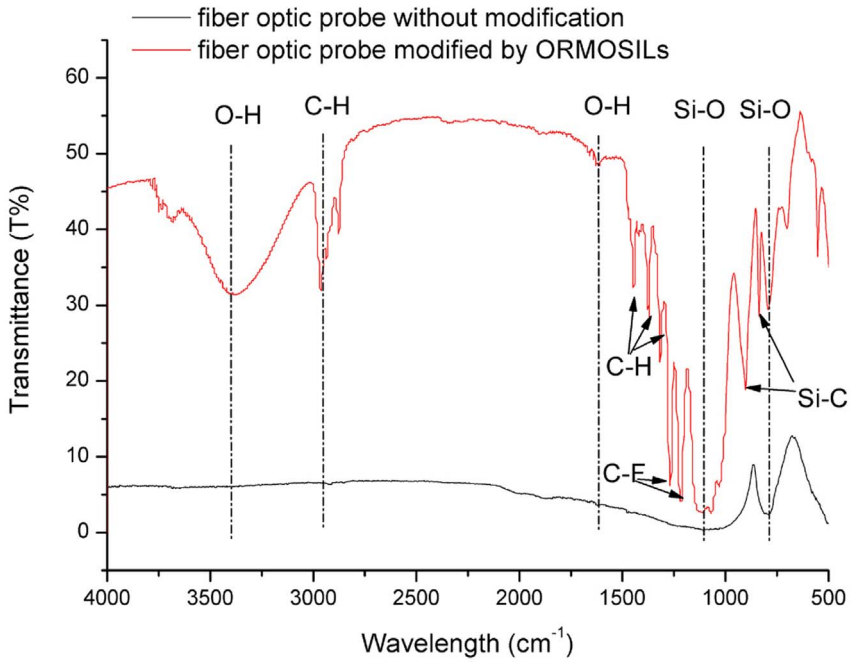

Figure 2 | **FTIR spectra of the bulk fiber optic probe (black) and ORMOSILs-coated probe (red).**

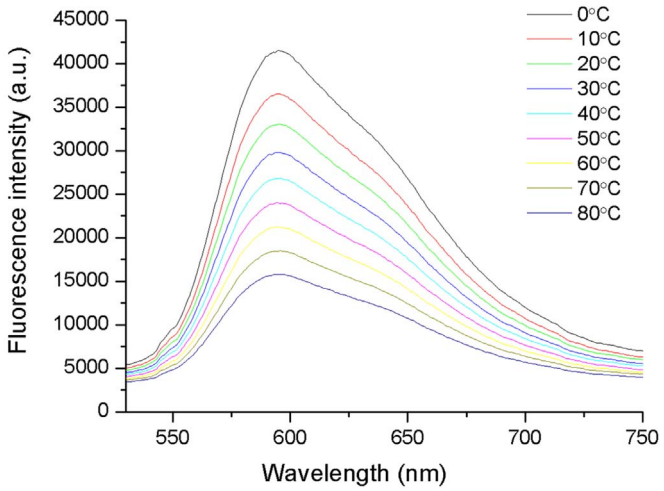

Figure 3 | Temperature-dependent PL spectra of the optode.

and n-propyltrimethoxysilane (n-propyl-TriMOS) and reaction with surface hydroxyl Si-OH of the fiber optic probe. Except for the peaks for asymmetric and symmetric stretching vibration of Si-O-Si bond, 3,442 cm^{-1}, 2,980 cm^{-1}, and 1,635 cm^{-1} correspond to physical absorption of water, stretching vibration of C-H bond, and surface hydroxyl of Si-OH, respectively, several other groups of strong peaks are also shown[20]. For example, 1,450, 1,375, and 1,317 cm^{-1} were corresponding to rocking vibration, wagging vibration, and twisting vibration of C-H for CH_2, respectively; 1,269 and 1,220 cm^{-1} correspond to asymmetric and symmetric stretching vibration of C-F bond, respectively; and 901 and 838 cm^{-1} correspond to vibration of Si-C bond[19,20]. These results indicate that the ORMOSIL-based film has been successfully modified onto the surface of the fiber optic probe.

Increasing temperature introduces higher probability of non-radiative transitions, thus decreasing the luminescence yield and the excited state lifetime[1,13]. To obtain temperature-dependent PL spectra of Ru complex, we link the single-multi mode fiber coupler with the QE65000 spectrometer (Ocean optics, USA). Figure 3 depicts the temperature-dependent PL spectra of the Ru complex. The PL intensity decreases dramatically as the suspension temperature is increased from 0°C to 80°C. A similar result was reported for the Ru complexes-based temperature sensor[1].

PL intensity changes versus temperature detected by using all-fiber structure based PL measurement system. Although the spectrometer can record the relationship between temperature and fluorescence intensity from Ruthenium (II) complex, achieving continuous and long-term temperature detection is difficult. To achieve this objective, an integrated all-fiber structure based PL measurement system (Figure 4) was developed for real-time detection of the sample temperature.

Figures 5A and 5B demonstrate the temporal temperature-dependent fluorescence signal curve and the relationships of PL intensity versus temperature, respectively. Figure 5A shows that with increasing temperature, the PL intensity diminishes monotonically from 0°C to 80°C, and the response to temperature is completely reversible, which indicates the great potential of our optode for fabricating of temperature-sensitive devices. The response of the optode to temperature is instantaneous and limited by heat transfer rates. After placing the tip of the fiber in a solution of a different temperature, the optode responded within 3 s (Figure S1). With the intensity of the strongest PL peaks at 0°C set as 1, the intensity at a higher temperature exhibits a proportional trend. The relationship between PL intensity and temperature is plotted in Figure 5B (squares), and it can be fitted approximately by an exponential decay function as follows:

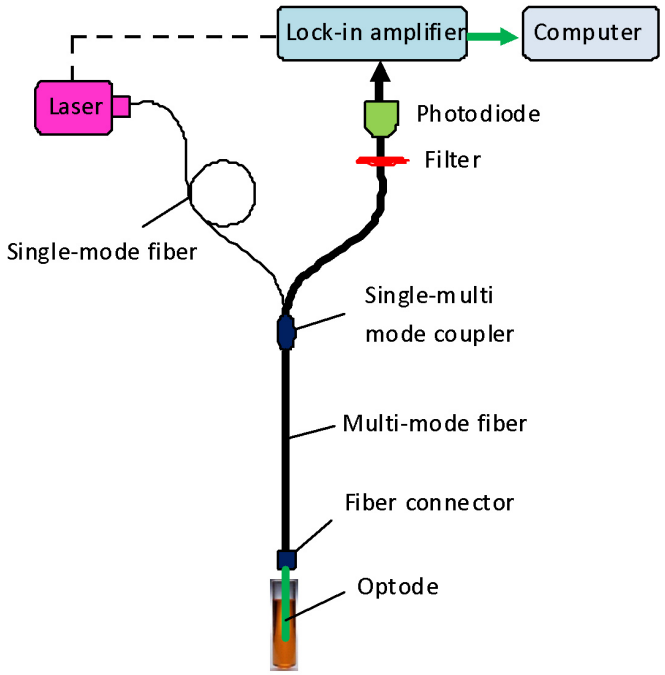

Figure 4 | Schematic diagram of the all-fiber structure based PL measurement system.

$$Y = 1.323^{\wedge}\exp(-T/113.726) - 0.363$$

When temperature is less than (or higher than) 50°C, an almost linear correlation was found between the fluorescence intensity and the temperature by using piecewise linear approximation. The slope of low temperature is higher than that of the high temperature. The linear dependence of fluorescence intensity on the temperature allows the use of a simple two-point calibration with minimal error when this point falls in the linear measurement range (e.g., 0°C to 50°C). From these results, we can obtain the sensitivity of the optode as 0.015°C based on a signal-to-noise ratio (S/N) of 3. The high sensitivity may contribute to the structure of optode, in which the Ruthenium complex can be excited twice, and the excited fluorescence can be reflected by the Al-based mirror. These conditions can enhance the detected fluorescence signals and improve the sensitivity of the system. The temperature-sensitive part of the optode is only Φ1 × 1 mm, which means that the presented optode can be used to detect a very small volume sample (<5 μL). Therefore, this optode can be used for temperature detection in microscale cells, channels and reactors. The Ruthenium complex of this optode was caged in a capillary and was not direct contact with other materials (e.g., O_2 or other materials that affect the quantum yield of Ru complexes), and the effect of the sample matrix can be excluded completely. Moreover, the detected samples are unaffected by the release of the Ruthenium complex of this optode. Therefore, the optode is non-toxic, which is very important for the temperature detection of biological and chemical samples.

Photostability of the optode. Laser was used to excite the Ruthenium complex, which means that photodegradation can be a critical issue even for indicators that possess good photostability in other conditions. Nevertheless, we have found that the presented optode exhibited surprisingly good photostability. No noticeable decrease in luminescence intensity (<5%) was observed after 24 h continuous in-situ detection. Furthermore, although the proposed optode performed daily measurements over 90 d of continuous analysis at 50°C, the luminescence intensity decreased by less than 7.2% only (data not shown). This photostability may be due to the following

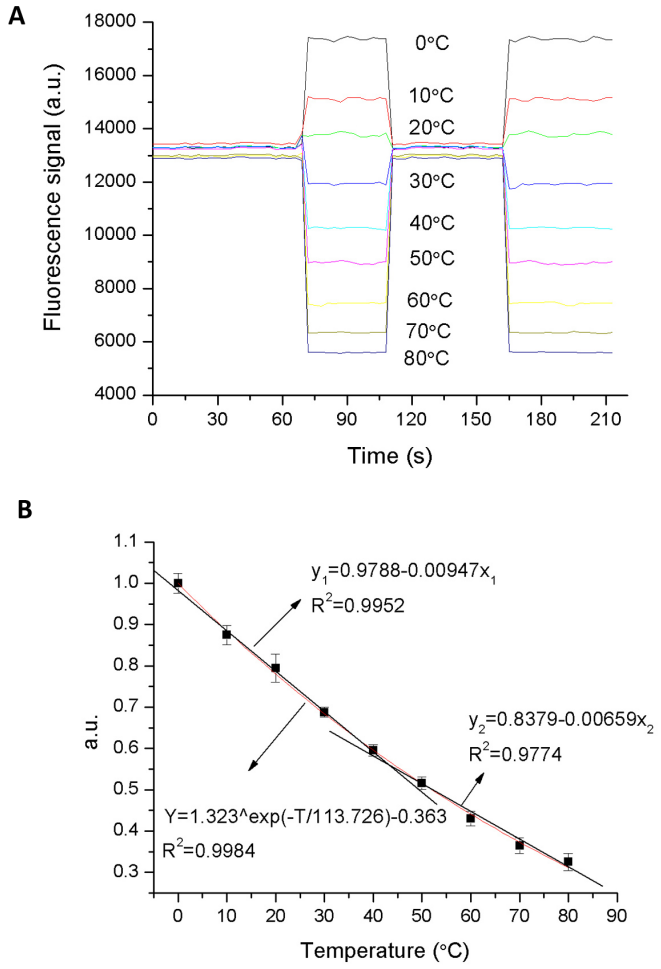

Figure 5 | (A) Signal change and reversibility of the temperature-sensitive optode; (B) Plots of the average PL intensity (black square) versus temperature. The red line is the fitted PL intensity as a function of temperature; the two black lines are linear correlations between fluorescence signal and temperature when temperature is less than or more than 50°C, respectively. The error bars represent standard deviations from the average values from three independent measurements.

reasons: First, the optimized optical structure of the optode makes possible the use of the minimum laser power, which is less than 0.5 mW at an output of MMF. Moreover, the structure of the optode and the all-fiber optical system can obviously increase the excited fluorescence and collected efficiency, and a high-pass filter was employed at the detector to block the fundamental excitation light that was elastically reflected and scattered back from the optode. Second, a digital lock-in amplifier was applied for the increase of the system's sensitivity by improving the S/N of the experimentally measured fluorescence signal. The digital lock-in amplifier, as a narrow band-pass filter, allows the signal to be measured to pass through while effectively removing most of the unwanted noise. To achieve this condition, the laser excitation was modulated at a reference frequency that induced fluorescence emission from Ruthenium complex at the same frequency. Then, the modulated signals detected at the photodiode were sensitively monitored using the digital lock-in technique, the parameters of which, such as time constant and sensitivity, were selected to maximize S/N at the given modulation frequency. Finally, the modulation excited light of the pulse laser benefits for the recovery of fluorescence molecules.

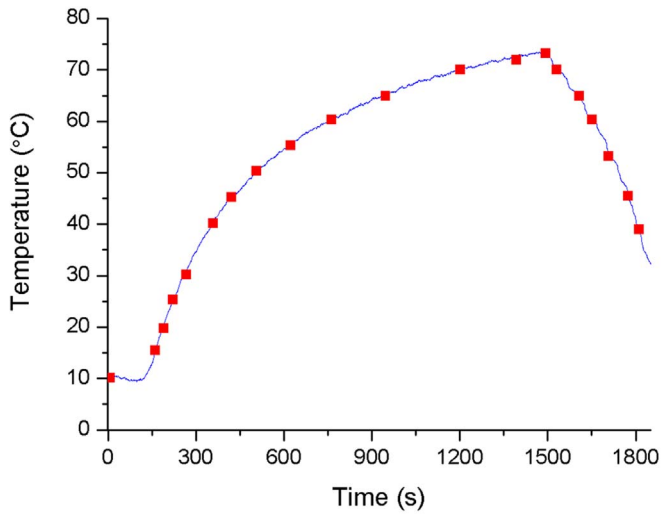

Figure 6 | Comparison between in situ and discrete temperature measurements. Red points indicate temperatures detected by the mercurial thermometer; the blue line indicates temperatures detected by the presented optode.

Comparison of in situ and discrete temperature measurements. Real-time in-situ temperature sensing is very important especially for micro-reaction process, polymerase chain reaction, and cellular physiology[2,6]. To evaluate the real-time in-situ temperature sensing capability of the optode, we conducted a 0.2 mL centrifugal tube-based (Φ6.2 × 24 mm) micro-reaction cell, which was immersed in a water bath. Then, 50 μL of water was added in the cell, the temperature of which could be regulated by using water-bath. The temperature of the water-bath was detected by using a mercurial thermometer, and the temperature of the micro-reaction was tested by the proposed optode. Figure 6 indicates excellent agreement between the in situ measurements and the discrete temperature values measured by the mercurial thermometer. The differences were typically 0.08°C to 0.15°C over the temperature range of 10°C to 70°C. These results show that the presented all-fiber sensing platform can be used for real-time in situ detection of the sample. The small size of the optode means that this platform can be used for continuous detection of a very small sample volume.

Discussion

A simple and compact optode has been developed for continuous and in-situ temperature detection in a very small volume sample. The transmission of excitation light and the collection and transmission of fluorescence are performed by a homemade single-multi mode fiber coupler, which provides the entire system with a simple and robust structure. The presented optode has several advantages over other optodes. First, the presented optode is non-toxic and matrix-effect-free because the Ruthenium complex of this optode was caged in a capillary and was not in direct contact with detected samples; such properties are essential for temperature detection in chemical, environmental or biological samples. Second, simply structure of the presented optode greatly improves the sensitivity and robustness of the system. Third, this optode has advantages of high photostability and chemical stability, a wide detection range, and thermal reversibility. Furthermore, the presented system can be applied to on-site and on-time rapid temperature detection of a very small volume sample with high sensitivity.

Methods

Materials and chemicals. TFP-TriMOS, n-propyl-TriMOS, ethanol (EtOH), and $[Ru(bpy)_3]^{2+}$ were purchased from Sigma-Aldrich (Steinheim, Germany). All other

reagents, unless otherwise specified, were supplied by Beijing Chemical Agents. The reagents were analytical grade and used without further purification.

Optode preparation. The ORMOSILs-based Ruthenium (II) complex was prepared as previously described with few modifications[18]. 1.2 mL TFP-TriMOS and 0.60 mL n-propyl-TriMOS were first mixed to form a precursor solution. Then, 1.5 mL EtOH, 0.08 mL of 0.1 M HCl, and 0.635 mL deionized water were added to catalyze the ORMOSIL reaction, and the mixture was sonicated for 1.5 h. 5 mM $[Ru(bpy)_3]^{2+}$ solution was prepared by dissolving $[Ru(bpy)_3]^{2+}$ in EtOH. The Ruthenium complex-doped sol solution was prepared by mixing 20 μL of 5 mM $[Ru\ (bpy)_3]^{2+}$ solution with an 80 μL TFP-TriMOS/n-propyl-TriMOS solution. This fluorinated xerogel-based mixture was sonicated for 15 min under room temperature. A step-index silica optical fiber probe (Chunhui Science & Technology Industrial Co., China) with a length of 10 cm and a diameter of 600 μm was cleaned by piranha reagents (concentrated H_2SO_4/H_2O_2 2 : 1), rinsed with ultrapure water, and dried in N_2. Next, the distal end of the probe was immersed into the fluorinated xerogel solution for 4 h under an inert atmosphere to coat a ORMOSILs-based film. The prepared probe was dried at least 5 d at RT. Finally, the probe was inserted into a capillary, which has a length of 6 mm, an internal diameter of 600 μm, and an external diameter of 1000 μm. The end of the capillary was sealed by a glass sheet deposited aluminum film, which will enhance the excited efficiency of the Ruthenium complex due to the reflected laser.

Instrument: all-fiber structure based PL measurement system. o confirm the performance of the fabricated optode, an all-fiber structure based PL measurement system was constructed as shown in Figure 4. A specific single-multi mode fiber coupler (SMFC) was used for transmitting excitation light and collecting and transmitting fluorescence. The SMFC was coaxially aligned with the ORMOSILs-based optode. To reduce photobleaching of the Ruthenium complex, a pulse fiber pigtailed laser diode (635 nm, 5 mW, 3 KHz) (BWT Beijing, China) was used as the excited light source. The excitation light was delivered to the optode through a multi-mode fiber (MMF; NA = 0.22, id./od. = 580 μm/600 μm) of SMFC. The fluorescence signal from the optode was collected by the same MMF. A long-pass filter (Semrock, USA) at 450 nm was employed at the detector part to reject the fundamental excitation light that was elastically reflected back from the sample surface. Subsequently, the collected fluorescence was monitored by photodiodes through digital lock-in detection. Reducing optical components and unnecessary optical alignment ensures that the all-fiber optical sensing platform can be suitable for rapid in-situ temperature detection of the sample.

1. Filevich, O. & Etchenique, R. 1D and 2D temperature imaging with a fluorescent ruthenium complex. *Anal. Chem.* **78**, 7499–7503 (2006).
2. Okabe, K. *et al*. Intracellular temperature mapping with a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy. *Nat. Comm.* **3**, 705 (2012).
3. Aigouy, L., Tessier, G., Mortier, M. & Charlot, B. Scanning thermal imaging of microelectronic circuits with a fluorescent nanoprobe. *Appl. Phys. Lett.* **87**, 184105 (2005).
4. Benninger, R. K. P. *et al*. Quantitative 3D mapping of fluidic temperatures within microchannel networks using fluorescence lifetime imaging. *Anal. Chem.* **78**, 2272–2278 (2006).
5. Shah, J. J., Gaitan, M. & Geist, J. Generalized temperature measurement equations for rhodamine b dye solution and its application to microfluidics. *Anal. Chem.* **81**, 8260–8263 (2009).
6. Wang, X. *et al*. Preparation of reversible colorimetric temperature nanosensors and their application in quantitative two-dimensional thermo-imaging. *Anal. Chem.* **83**, 2434–2437 (2011).
7. Jaque, D. & Vetrone, F. Luminescence nanothermometry. *Nanoscale* **4**, 4301–4326 (2012).
8. Brites, C. D. *et al*. Luminescent molecular thermometer for long-term absolute temperature measurements at the nanoscale. *Adv. Mater.* **22**, 4499–4504 (2010).
9. Hu, S. H., Chen, Y. W., Hung, W. T., Chen, I. W. & Chen, S. Y. Quantum-dot-tagged reduced graphene oxide nanocomposites for bright fluorescence bioimaging and photothermal therapy monitored in situ. *Adv. Mater.* **24**, 1748–1754 (2012).
10. Peng, H. *et al*. Luminescent europium(III) nanoparticles for sensing and imaging of temperature in the physiological range. *Adv. Mater.* **22**, 716–719 (2010).
11. Gan, Z., Wu, X., Zhang, J., Zhu, X. & Chu, P. K. In situ thermal imaging and absolute temperature monitoring by luminescent diphenylalanine nanotubes. *Biomacromolecules* **14**, 2112–2116 (2013).
12. Hsia, C., Wuttig, A. & Yang, H. An accessible approach to preparing water-soluble Mn^{2+}-doped (CdSSe)ZnS (Core) shell nanocrystals for ratiometric temperature sensing. *ACS Nano* **5**, 9511–9522 (2011).
13. Lee, K. W. *et al*. Photophysical properties of tris(bipyridyl)ruthenium(II) thin films and devices. *Phys. Chem. Chem. Phys.* **5**, 2706–2709 (2003).
14. Mármol, J., Filevich, O. & Etchenique, R. A ruthenium-rhodamine complex as an activatable fluorescent probe. *Anal. Chem.* **82**, 6259–6264 (2010).
15. Feng, W., Zhou, N., Chen, L. & Li, B. An optical sensor for monitoring of dissolved oxygen based on phase detection. *J. Opt.* **15**, 055502 (2013).
16. Pulido, C. & Esteban, ó. Tapered polymer optical fiber oxygen sensor based on fluorescence-quenching of an embedded fluorophore. *Sens. Actuators B* **184**, 64–69 (2013).
17. Atkins, G. R. & Charters, R. B. Optical properties of highly fluorinated and photosensitive organically-modified silica films for integrated optics. *J. Sol–Gel. Sci. Technol.* **2**, 919–923 (2003).
18. Campostrini, R., Ischia, M. & Carturan, G. Sol-gel synthesis and pyrolysis study of oxyfluoride silica gels. *J. Sol-Gel. Sci. Technol.* **23**, 107–117 (2002).
19. Lamber, J. B., Shurvell, H. F., Lightner, D. A. & Cooks, R. G. Organic structural analysis. Prentice Hall, London, 1998.
20. Xiong, Y., Zhu, D., Chen, S., Peng, H. & Guan, Y. A fiber-optic evanescent wave O_2 sensor based on Ru(II)-doped fluorinated ORMOSILs. *J. Fluoresc.* **20**, 269–274 (2010).

Acknowledgments

This research was financially supported by the National Natural Science Foundation of China (21077063, 21277173), the National Instrument Major Project of China (2012YQ3011105), and the Basic Research funds in Renmin University of China from the central government (13XNLJ01).

Author contributions

F.L. performed all the experiments, and wrote the manuscript. H.-C.S. and F.L. designed all the experiments and finalized the preparation of the manuscript. F.L. designed and managed the project. All the authors discuss the results and commented on the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Long, F. & Shi, H.C. Simple and compact optode for real-time in-situ temperature detection in very small samples. *Sci. Rep.* **4**, 5009; DOI:10.1038/srep05009 (2014).