A Photochromic Sensor Microchip for High-performance Multiplex Metal Ions Detection

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Current multi-analytes chips are limited with requiring numbers of sensors, complex synthesis and compounds screen. It is expected to develop new principles and techniques to achieve high-performance multi-analytes testing with facile sensors. Here, we investigated the correlative multi-states properties of a photochromic sensor (spirooxazine), which is capable of a selective and cross-reactive sensor array for discriminated multi-analytes (11 metal ions) detection by just one sensing compound. The multi-testing sensor array performed in dark, ultraviolet or visual stimulation, corresponding to different molecular states of spirooxazine metal ions coordination. The facile photochromic microchip contributes a multi-states array sensing method, and will open new opportunities for the development of advanced discriminant analysis for complex analytes.

There is an increasing need of convenient, rapid, sensitive and high-throughput sensors for target multi-analytes identification in the environmental monitoring1–4, aliment safety5,6, clinical diagnoses7–14, and so on15,16. Sensor arrays are a verified valid detection method in the area of multi-analytes testing and high-throughput analysis17–20. Currently, a graphene-based ensemble aptamers were exploited to recognize molecular or cellular targets discriminatively17. Cui et al. developed a sensor array assembled from polyionic liquid inverse opaline microspheres to test five anions18. Generally, the sensor array performed the correlative differential analyzing needs large numbers of serial compounds or complicated compound with several recognition groups as sensor, which involves complicated chemical synthesizing and valid compounds screening. Examples include adopting six various 8-hydroxy-quinoline derivants to realize metal ions detection19, designing sensor combining four hydrophobic recognition groups to take molecular diagnostics20, seven sensors utilized a common calix[4]pyrrole receptor to detect 14 carboxylates21. Although the serial sensors own high sensitivity, the high production cost and challenges regarding valid compounds screen restrict their practical applications. These facts call for a reliable sensor to alleviate these concerns for general high-performance multi-analytes detection. Here, we adopt a photochromic material, spirooxazine, to multi-testing sensor array, and utilize the multi-states of the sensing array under stimulations acting the selective and cross-reactive sensing (Fig. 1). This photochromic sensor microchip can actualize eleven various metal ions detection with only one facile sensor, which offers a new strategy to design and develop high-performance multi-testing sensor array.

Spiropyran and spirooxazine (SP), which respond to different external stimulations, such as light, proton and metal ions to undergo reversible structural interconversion accompanied the change in photophysical properties, are of interest in the emerging area of logic gates and communication networks24–26, molecular switches and unit27,28, data storage29, metal ions detection and other fields30–37. With different light stimulations, spiropyran and spirooxazine interconvert among the closed form to merocyanine form (MC). The merocyanine spiropyran/spirooxazine can combine with proton or metal cations to form metallic merocyanine (MMC), as shown in Fig. 224–26,30–37. Performing the reversible reactions between closed, MC and MMC forms of spiropyran/spirooxazine, different ions strength and light stimulations have the differential inductive effect. It gives abundant chemical information in forms of interconvert processing, which can be adopted to high-throughput and complex analysis. Since fluorescent detection owns the significant advantages of high-sensitive, quick-response and non-contacting, we choose a commercial spirooxazine: 1,3,3-trimethylindolinonaphthospirooxazine to build a photochromic sensor microchip for high-performance multiplex metal ions detection.
Results

Fig. 3 displays the typical fluorescence spectra of spirooxazine in ethanol (1.0 mM) response with metal ions in different light stimulations. Spirooxazine combining with Al\(^{3+}\) has obvious fluorescence increasement, ultraviolet (UV) light irradiation grows the 533 nm fluorescence and visible (Vis) light irradiation drastically increases the fluorescent peak of 435 nm. In the case of spirooxazine response with Co\(^{2+}\), Vis light stimulation increases the peaks of 435 and 533 nm of the fluorescent spectra. Cu\(^{2+}\) has the strong coordinating ability with spirooxazine/spiropyran and results in the closed form ring-open. The spirooxazine-Cu\(^{2+}\) coordination generates a medium fluorescence. UV and Vis irradiation further enhance the fluorescence at 533 nm. Spirooxazine-Zn\(^{2+}\) coordination dose not contribute the obvious fluorescence when prime stain and UV irradiation. The electron transfer from Zn\(^{2+}\) to organic ligand under Vis irradiation contributes a weak fluorescence emission. The change of fluorescence spectra of spirooxazine combined with Al\(^{3+}\), Co\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\) under dark, UV and Vis irradiation are focus at the wavelength of 435 nm and 533 nm because of the light stimulations induce the merocyanine of the spirooxazine-metallic coordinations partly convert to open or closed form. Furthermore, when this sensing system was adopted in cross-reactive array sensing, fluorescent signals were collected in the same condition at the same time, which will avoid error from excitation light source glint, detector fatigue. Accurate chemical difference was present in this sensing array system. The phenomena that spirooxazine-metallic coordinations reveal various fluorescent spectra in different light stimulations demonstrate the sensing molecular spirooxazine can provide multiple fluorescent signals, which will contribute to the photochromic sensor microchip selective and cross-reactive testing.

Based on the fluorescent enhancement or shift of different spirooxazine-metallic coordination states, we designed a microchip adopting spirooxazine as the only one sensor, analysing the fluorescent signals to realize multiple metal ions detection. To generate the cross-reactive SP microchip, we carried out 1.0 mM 1, 3, 3-trimethylinelinonaphthospirooxazine and thermoplastic polyurethane Tecoflex\(^{\copyright}\) (0.5% PU in Ethanol) solution (200 nL) pipetted onto each pixel of the microchip. Here we used PU as the interface medium for the sensing in each pixel. PU is a block polymer with terephthalic acid units and ethylene glycol units, which is solvable in ethanol and swellable in water. On this microchip, AlCl\(_3\), CaCl\(_2\), CdCl\(_2\), CrCl\(_3\), CuCl\(_2\), FeCl\(_2\), HgCl\(_2\), MgCl\(_2\), NiCl\(_2\), and ZnCl\(_2\) (1.0 mM in water, 200 nL, pH 5) and pH 5 control aqueous solutions (HCl-NaCl) were spotted on corresponding pixels in 12 rows respectively. The fluorescence responses of the photochromic spirooxazine microchip to the presence of 11 metal ions (Al\(^{3+}\), Ca\(^{2+}\), Cd\(^{2+}\), Co\(^{2+}\), Cr\(^{3+}\), Cu\(^{2+}\), Fe\(^{3+}\), Hg\(^{2+}\), Mg\(^{2+}\), Ni\(^{2+}\) and Zn\(^{2+}\)) were recorded in 6 channels (CH 1: 450 nm, CH 2: 480 nm, CH 3: 505 nm, CH 4: 535 nm, CH 5: 570 nm and CH 6: 605 nm, with UV 365 nm excitation). The colours were generated by
superimposing of the equally weighed images corresponding to RGB channels. Dark, UV and Vis stimulation resulted in the spirooxazine microchip displaying various fluorescent signals. For better comparing the fluorescent difference of dark, UV and Vis process, rows from each light irradiation steps were selected to constitute Fig. 4. The fluorescent image in Fig. 4b displays obvious different fluorescence intensity and shift for each row, which will contribute more information for high-performance multi-testing.

The discriminatory capability of the photochromic SP microchip can be performed using multivariate analysis. Linear discriminant analysis (LDA) was used to evaluate the similarities between the data corresponding to the same cluster by introducing the group classification. LDA provides a graphic representation useful to gain an insight into the clustering of the response data, and to calculate classification accuracy. Fig. 5a, b represent the LDA score plot of the first three dispersion factors (F1–F3) describing of the total variance, which displays the clear clustering of the data. Obviously, the LDA score plot shows clustering for all 12 samples (7 trials each, 1.0 mM). It gives the 100% correct clustering of the eleven metal ion samples and one control sample water pH 5 (Table S1, Supporting Information [SI]). The clusters were separated in the space of differential fluorescent peak (435 or 533 nm) distribution orientation. Furthermore, we also adopted hierarchical clustering analysis (HCA) to carry out multivariate analysis. HCA performs dimensionality reduction analysis to investigate the similarity clustering of the analytes. In this work, we defined the cluster by Ward’s (minimum variance) method, which takes into consideration the minimum amount of variance between the samples. The HCA result shows a dendrogram of euclidean distance between 84 samples with ward linkage in Fig. 5c and Fig. S1. HCA graphical output displays three major groups, which indicates fluorescent variation trend of the different states of spirooxazine-metallic coordination. Overall evaluating the LDA and HCA, the results reflect the original information of sensing, which indicates subtle and accurate details for the photochromic SP microchip sensing various metal ions. The spirooxazine-metallic responses focus on the two major peaks of 435 and 533 nm on fluorescence spectra. The different fluorescence intensity ratios between 435 and 533 nm reveal various classification contributions of photochromic SP microchip in different light stimulations.

**Discussion**

To evaluate the classification efficiency of the photochromic SP microchip with various states, we explored the LDA of SP array in dark, UV or Vis stimulation state separately (Table S2–S4). None of the single state of SP array can result in a completely correct classification individually. The LDA results showing in Table S5 displays the near and confused spatial distribution of the clusters in each single state of SP array, which reveals the small differential of fluorescent signals of the metal ions in these analysis systems. In details, the SP sensing array in dark state can discriminate Al³⁺, Cr³⁺, Cu²⁺, Fe²⁺, Hg²⁺, Mg²⁺, Ni²⁺ and Zn²⁺, and cannot correctly analysis Ca²⁺, Cd²⁺, Co²⁺ and pH = 5. UV state does not improve the general accuracy than dark state, but it increases the accurate rate for Ca²⁺ identification and help to distinguish misclassified Co²⁺ from control (pH = 5). In the case of Vis state, the microchip generates the clear identification of Al³⁺, Cd²⁺, Co²⁺, Cr³⁺, Cu²⁺, Fe²⁺, Hg²⁺, Mg²⁺, Zn²⁺ and pH = 5, only has deficiency at Ca²⁺ and Ni²⁺. The three
Figure 4 | (a) Fluorescent image and (b) 3D representation of the integrated fluorescence intensity of the photochromic SP microchip constitute from rows from each light irradiations process of the photochromic microchip. The Fluorescent image of photochromic microchip spotted by metal ions (1.0 mM in water, 200 nL), which was recorded in 6 different channels. The colours were generated by superimposing of the equally weighed images corresponding to RGB channels.

Figure 5 | Fluorescent discriminant analysis of 11 metal ions on SP multi-states microchip and rational analysis. (a, b) Graph of LDA result shows a clear clustering of the 11 metal ions analytes and its corresponding magnified image. LDA reflects analytes specific fluorescent enhancement at 435 nm or 533 nm due to various metal electropositivity. The gathering of the clusters demonstrates the good repeatability of the SP multi-states microchip for each metal ion response. (c) HCA gives the similarity clustering of the analytes based on the fluorescent variation trend of the spirooxazine-metallic coordination in three states.
states are highly complementary in distinguishing metal ions. It guarantees the integrated SP multi-states array to perform the 100% correct clustering of the eleven metal ion samples with one control sample.

The key point of sensor array successful in multi-targets analysis is to provide enough differential and correlative signals. However, most single sensing molecule lacks enough differential signals to correctly cluster of the multi-analytes. Previous researches usually focused on developing serial sensors to get correlatively differential messages\(^7\)\(^{-21}\). The strategies usually are preparing sensor array with serial sensor compounds to plus and fold the correlative response difference, or synthesizing special and complex sensor compound to generate obviously discriminated response. Here, the single photochromic sensing compound gives a strong correlativity between the configurations interconverting under different light stimulations. The differential states under dark, UV and Vis stimulation generate differential and correlative signals, which perform as three serial sensors adopted in this sensing array and contribute to perform a correct discriminant analysis. By the well-known Kolmogorov’s law of the iterated logarithm\(^{22}\), the relation of multi-targets analysis successful probability \(P(x_{ij})\) and participant observation states \(n_i\) can be deduced as (For the detailed deduction process, please see Supporting Information):

\[
P(x_{ij}) \geq \int \left( \frac{\Delta}{\bar{n}^i} - \frac{2}{\bar{n}^i} \max_{1 \leq i \leq n} \sqrt{\frac{\log \log n_i}{n_i}} \right) f(t) dt
\]

There are many factors contribute to the probability of success of multi-targets analysis, here we focus on the influence of \(n_i\). The \(x_{ij}\) presents the classification of success. The mathematical deduction suggests the universality of the strategy applied in multi-targets analysis. It can be simply described as a monotonically increasing function. That is meaning the calculated probability of success obviously increases when the observation’s number increases, which is well in agreement with experiment results. The multi-states analysis method guarantees the generated signals cross-reactive and differential, which realizes high-performed multi-analytes identification on a single sensing compound array, and avoids the complex process of serial synthesis and valid compound screening.

Encouraged by the results above, we explored the utility of the spirooxazine multi-states sensor array by exploring a potential application: identification of mineral and purified water (Milli-Q water) based on their cation content as shown in Fig. 6. Table 1 lists

![Figure 6](https://www.nature.com/scientificreports) LDA score plot corresponding to the response of the SP microchip to 16 kinds of natural mineral water comes from various countries. The data set contains 16 brands and 1 control, 15 trials each. LDA shows 100% correct classification for all water brands.

| Mineral Water     | Ca (mg/L) | Mg (mg/L) | pH | Mineral Water     | Ca (mg/L) | Mg (mg/L) | pH |
|-------------------|-----------|-----------|----|-------------------|-----------|-----------|----|
| Baby Bella        | 53.3      | 14.9      | -  | Evian             | 80        | 26        | 7.2|
| Fiji              | 17.5      | -         | 7.7| Highland          | 40        | 10.1      | 7.8|
| Nong Fu Spring    | 40        | 5         | 7.3| Perrier           | 160       | 4.2       | -  |
| S. Antonio        | 32.4      | 5.3       | 7.9| Sam Da Soo        | 2.9       | 1.9       | 7.7|
| San Benedetto     | 50.3      | 30.8      | 7.52| Source Naturelle  | 54        | 4.5       | 7.5|
| Source Pure Red   | 54        | 4.5       | 4.7| SPA               | 4.5       | 1.3       | 6  |
| Teleno            | 6         | 2.2       | -  | Volvic            | 11.5      | 8.0       | 7  |
| Vswp              | 45        | 51        | -  | Waiwera           | 12        | 2.6       | 7.6|

Table 1 | Metal Ion content for different brands of mineral water samples. The samples contain different kinds and concentrations of metal ions
Ca$^{2+}$ and Mg$^{2+}$ contents for all of the mineral water brands. The pH levels of most brands are in the range of 6–7.8 (except the Soursy pure red with pH = 4.7), where the sensor array presents a rather flat response. The list is clear that all 16 commercial potable water samples contain different concentrations of cations. LDA analysis shows a complete and clear 100% correct classification for all 255 trials (Figure S6). The study demonstrates that the photochromic SP microchip detection of metal ions can be generated into metal ions mixtures. Furthermore, we also processed the photochromic SP microchip for detecting metal ions in human serum (protein removed), and the LDA result displays 100% classification accuracy (Fig. S2, Table S7). The result suggests its potential applications in complex physiological environment.

In conclusion, utilizing interconversion of photochromic materials, we developed a high-performance multi-analyses sensor array with a single compound. Significantly, this sensor array is capable of identification of 11 metal ions, based on multi-states of spirooxazine in UV and Vis irradiances. The successful discriminating of various natural mineral water samples proves the photochromic SP microchip practical application in mixed and complicated analysis. It contributes a new idea of stimulated (photo-, electro-, magnet-, thermo-, chemo-, etc) conversion sensor array for multi-testing, which will have implications in applications in chemical, biological or medicinal samples testing. The future complex sample detections for pollution monitoring, aliment safety and clinical diagnoses will be facile, efficiency and low-cost with the high-performance sensing and analysis method.

**Methods**

**Fluorescence testing.** A UV LED lamp with the wavelength of 245 nm was used as the UV illumination source. Fluorescence measurements were performed in a Hitachi F-4500 Fluorescence Spectrophotometer. The absorption spectra were measured with a fiber optic UV-Vis-IR spectrometer (Ocean Optic HR 4000 CG).

**Microchip sensing.** 1.0 mM 1, 3-trimethylindolino-naphthospirooxazine and thermoplastic polyurethane Tecoflex® (0.5% PU in Ethanol) solution (200 nL) was pipetted onto each pixel of the microchip. The microchip was performed to the multi-analyses testing (11 metal ion plus the pH = 5 control solution). On the microchip, AlCl$_3$, CaCl$_2$, CdCl$_2$, CoCl$_2$, CrCl$_3$, CuCl$_2$, FeCl$_3$, HgCl$_2$, MgCl$_2$, NiCl$_2$ and ZnCl$_2$ (1.0 mM in water, 200 nL, pH = 5) and pH = 5 control aqueous solutions (HCl–HClO$_4$) were spotted on corresponding pixels respectively. The fluorescence responses of the microchip to different metal ions were recorded by fluorescence scanner (ChampChemmi Professional ™) in six channels (CH1: 450 nm, CH2: 480 nm, CH3: 505 nm, CH4: 535 nm, CH5: 570 nm and CH6: 605 nm, with 365 nm UV light excitation). The data processing consisted on the integration of the fluorescence intensity per pixel of the microchip before and after the solutions of metal ions spotted.

**Data analysis.** The statistical multivariate methods, LDA and HCA were routinely used to interpret and evaluate the responses from cross-reactive sensor arrays, providing a graphical output useful to gain an insight into the clustering of the response data, and calculate classification accuracy. The metal ion spots were formed on microchip and record in six channels by fluorescence scanner. The LDA was carried out using SYSTAT® v12.02.00 and the HCA was carried out using Minitab® v16.1.1.0.

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Author contributions
Y.H., F.L. and Y.S. conceived and designed the experiments. Y.H., F.L. and M.Q. performed the experiments. Y.H., F.L., C.Y. and W.R. analysed the data and wrote the manuscript. All authors reviewed the manuscript.

Additional information
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