Multicolor Fluorescence Detection-Based Microfluidic Device for Single-Cell Metabolomics: Simultaneous Quantitation of Multiple Small Molecules in Primary Liver Cells

Qingling Li, Peilin Chen, Yuanyuan Fan, Xu Wang, Kehua Xu, Lu Li,* and Bo Tang*

College of Chemistry, Chemical Engineering and Materials Science, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Provincial Key Laboratory of Clean Production of Fine Chemicals, Shandong Normal University, Jinan, 250014, P.R. China

ABSTRACT: Single-cell metabolomics can be used to study cell diversity and how cells respond to environment. There is an urgent need to develop effective detection methods for single-cell metabolomics. Microchip electrophoresis with laser-induced fluorescence detection (MCE-LIFD) is a powerful tool to detect metabolites at the single-cell level. However, the existing one-laser excitation and one-color fluorescence collection in MCE-LIFD is not sufficient for the simultaneous detection of multiple small molecules with wide variations in their fluorescence excitation and emission spectra. In this manuscript, we describe a multicolor fluorescence detection-based microfluidic device (MFD-MD) for single-cell metabolomics research. We selected primary liver cells from acute ethanol-stimulated mice as the model cells and hydrogen peroxide (H$_2$O$_2$), glutathione (GSH), and cysteine (Cys) as representative small-molecule metabolites for single-cell analysis. The microfluidic chip enabled accurate single-cell manipulation and effective electrophoresis separation. The new multicolor fluorescence detection permitted simultaneous analysis of H$_2$O$_2$, GSH, and Cys. Ethanol exposure induced an increase in H$_2$O$_2$ and a decrease in GSH and Cys. Obvious cell heterogeneity was observed. These results provide insights regarding the intracellular oxidative/antioxidative molecular mechanism in response to external stimuli. The MFD-MD provides a new opportunity for simultaneous single-cell analysis of multiple metabolites.
and three optics channels for the green, red, and near-infrared fluorescence collection. These components can be adapted to wide spectral detection of multiple small molecules. To demonstrate the application of this system in single-cell metabolomics, we performed a simultaneous analysis of H$_2$O$_2$, GSH, and Cys in single liver cells after acute ethanol stimulation and silymarin protection (Scheme 1). Under certain conditions, such as acute and chronic ethanol exposure, pollution and hypoxia, reactive oxygen species (ROS) production is enhanced, and the level of antioxidants is reduced in cells. The imbalance between ROS production and antioxidant defense is referred to as oxidative stress. As an essential oxygen metabolite in cells, hydrogen peroxide (H$_2$O$_2$) is one of the most important ROS molecules generated in oxidative stress. H$_2$O$_2$ can cause oxidative protein modifications and lipid peroxidation. Additionally, H$_2$O$_2$ is present at much higher physiological levels than other ROS in cells and is closely related to the degradation and formation of other ROS, such as superoxide (O$_2^-$) and hydroxyl radical (•OH). By contrast, as important antioxidants, glutathione (GSH) and cysteine (Cys) play crucial roles against the toxicity of ROS. GSH is the most abundant intracellular thiol and a major cellular antioxidant. It plays a central role in combating oxidative stress and maintaining redox homeostasis, which is pivotal for cell growth and function. Cys is the major limiting precursor for GSH biosynthesis. As an essential intercellular thiol-containing amino acid, Cys is also involved in protecting proteins from oxidation by ROS. Because of the representative effects of H$_2$O$_2$, GSH, and Cys in oxidative stress, the simultaneous detection of these metabolites in cells in response to external stimuli is significant to further elucidating intracellular oxidant/antioxidant molecular mechanisms. In particular, single-cell analysis is necessary to address heterogeneity, which may be obscured by cell-population detection. In this study, primary liver cells from acute ethanol-induced mice were selected as model cells. After intracellular H$_2$O$_2$, GSH, and Cys reacted with their fluorescent probes FS (H$_2$O$_2$ probe) and Cy-3-NO$_2$ (GSH and Cys probe), the cells were introduced into a microchip and subsequently lysed. The intracellular contents were separated by microchip electrophoresis, and the three-color fluorescence signals corresponding to H$_2$O$_2$, GSH, and Cys were obtained using the MFD-MD.

**EXPERIMENTAL SECTION**

**Reagents and Materials.** All chemicals and solvents were of analytical grade. Water was purified with a Sartorius Arium 611 VF system (Sartorius AG, Germany). Bis (p-methylenenesulfonylate) dichlorofluorescein (FS) and Cy-3-NO$_2$ probes were synthesized in our laboratory. Glutathione (GSH), Cysteine (Cys), Dulbecco’s phosphate-buffered saline (D-PBS, Ca$^{2+}$/Mg$^{2+}$-free), and Hank’s balanced salt solution (D-hanks, phenol red, Ca$^{2+}$/Mg$^{2+}$-free) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO). Silymarin was purchased from Macklin reagent (Shanghai, China). The 40 μm cell strainer was purchased from Biologix (Lenexa, KS). The 8-week-old male Kunming mice (KM mice) were purchased from Shandong University Laboratory Animal Center. Stock solutions of FS (5.0 mM) and Cy-3-NO$_2$ (1.0 mM) were, respectively, prepared in dimethyl sulfoxide (DMSO) and acetonitrile and then were stored at −20 °C in darkness for further use. Stock solutions of H$_2$O$_2$ (1.0 mM), GSH (10 mM), and Cys (10 mM) were all prepared in ultrapure water. Phosphate-buffered saline (PBS buffer) used for electrophoretic migration were prepared by dissolving appropriate amount of Na$_2$HPO$_4$ and NaH$_2$PO$_4$ in ultrapure water. All solutions were filtered through a 0.22 μm nylon syringe filter before being added into the chip.

**Standard Sample Preparation.** Standard H$_2$O$_2$ was labeled by FS through mixing of different concentrations of H$_2$O$_2$ (500 μL) and 20 μM FS (500 μL) at 37 °C for 60 min. Standard GSH or Cys were labeled by Cy-3-NO$_2$ through mixing of different concentrations of GSH or Cys (500 μL) and 40 μM Cy-3-NO$_2$ (500 μL) at 37 °C for 60 min, respectively.

**Multicolor Fluorescence Detection-Based Microfluidic Device.** Multicolor fluorescence detection-based microfluidic device (MFD-MD) was built up to simultaneously detect the fluorescent signals from H$_2$O$_2$, GSH, and Cys (H$_2$O$_2$ emission, λ$_{max}$, 525 nm; Cys emission, λ$_{max}$, 755 nm; GSH emission, λ$_{max}$, 805 nm) in single cells. The schematic diagram of this device is shown in Figure 1. It consists of a glass microchip, a versatile programmable four-path-electrode power supply (PEPS), a new multicolor fluorescence detector, a data acquisition system, and a personal computer. The glass microchip has five reservoirs, all with 3 mm diameters and 1.5 mm depths. The auxiliary channel with 5 mm length intersected the sample loading channel (SC) 150 μm away from the crossing C (on the sample waste channel side) and formed a 45° angle with the sample loading channel. All channel cross sections had a nearly rectangular structure (70 μm width × 25 μm depth). A programmable four-path-electrode power supply (PEPS) is used to control the voltage outputs between the reservoirs on microchip. The new MFD-MD adopted a design containing two-laser excitation and three-channel fluorescence detection, combining with a laser-focusing microscopic observation mode. The two-laser excitation employed a 10 mW diode-pumped solid-state (DPSS) laser operating at 473 nm and a 15 mW DPSS laser operating at 730 nm (CNI Optoelectronics Tech. Co., Ltd. Changchun, China). The 730 nm laser beam and 473 nm laser beam reflected by the first reflector (M-1, Thorlabs) were combined through a
dichroic laser beam combiner (DLBC, Semrock) with a 350–488 nm reflection bandwidth and a 502–950 nm transmission bandwidth. The combined beams successively passed a beam expander (Thorlabs) and a dual-edge dichroic beam splitter (DEDBS, Semrock) with 440–488 nm and 600–735 nm reflection bandwidth and 500–580 nm and 740–900 nm transmission bandwidth, then focused the center of the separation channel about 33 mm downstream from the cross C with a 50X, 0.42 NA microscope objective (CVI, Melles Griot), forming a diffraction limited laser spot. The emitted fluorescence was collected by the same objective, penetrated through the same dichroic splitter DEDBS, and then was split into two light paths by the second single-edge dichroic beam splitter with a 350–596 nm reflection bandwidth and a 612–950 nm transmission bandwidth (SEDBS-1, Semrock). The reflected green fluorescence from H2O2 passed the first lens (L-1, Thorlabs) and was finally detected by PMT-1 (H9306-03, Hamamatsu). The transmitted light was split into a red light path and a near-infrared light path by the second single-edge dichroic beam splitter with 650–778 nm reflection bandwidth and 785–900 nm transmission bandwidth (SEDBS-2, Semrock). The reflected red fluorescence from GSH was reflected by the third reflector (M-3, Thorlabs) and passed the third lens (L-3, Thorlabs) and was finally detected by PMT-3 (H9306-03, Hamamatsu). Adjustable pinholes (P-1, P-2, and P-3, National Aperture) and band-pass filters (BP-1, 515–535 nm; BP-2, 745–765 nm; and BP-3, 795–815 nm; Semrock) positioned between the lens and the PMTs for spatial and spectral filtering. The laser-focusing observation was performed using a CCD (ec0655CVGE, SVS-Vistek, Co., Ltd) after pitching the second reflector (M-2, Thorlabs) in transmission light path of the dual-edge dichroics beam splitter by controlling the switch. The focusing of the laser on the center of the separation channel could be observed in real time. A high signal-to-noise ratio was obtained by optimizing the distance between the objective and the separation channel and the pore size of the pinhole (P-1, 100 μm; P-2, 200 μm; P-3, 200 μm). The focusing spot of the laser on the separation channel was improved to approximately circular by adding a beam expander (BE). To improve the detection sensitivity, band-pass filters were adjoined to the pinholes, and the distance between the PMTs and the pinholes was set to a maximum of 60 mm.

**Cell Sample Preparation.** All the animal procedures were carried out according to the Principles of Laboratory Animal Care (People’s Republic of China) and the Guidelines of the Animal Investigation Committee, Biology Institute of Shandong Academy of Science, China. The 8-week-old male Kunming mice were maintained under normal conditions with a 12 h light and dark cycle and allowed free access to water and standard lab chow. The mice were randomly divided into a control group, an ethanol-treated group, and a silymarin-treated group. The ethanol-treated group mice received 60% (v/v) ethanol in water, (4.7 g per kg BW) every 12 h for a total of three doses. The silymarin-treated group mice received silymarin (200 mg per kg BW) 1 h after each administration of ethanol. At the same time, the control group received an equal volume of water. The ethanol-treated group could be induced to significant acute liver injury as described in previous study. At 4 h after the last dosing, mice were sacrificed. Whole liver was immediately collected for the preparation of liver cells suspension. The liver tissue was dissociated into single cells by gentle grinding through a 40 μm cell strainer. The cell suspension was washed by centrifugation at 1500 rpm for 10 min and was resuspended in culture medium three times. Then the cells were incubated with 20 μM FS and 20 μM Cy-3-NO2 at 37 °C for 60 min. After that, the cells were washed with DPBS three times to remove the excessive probe. Finally, the cells were resuspended in 20 mM PBS (pH 7.4) with a density of 5 × 10^5 cells/mL. To get cell extract, the cells incubated as described above were disrupted with a BILON92-III ultrasonic disintegrator (Shanghai BiLon Materials Inc.). Then the cell homogenate was centrifuged at 15 000 rpm for 30 min using a speed refrigerated centrifuge (Sigma 3K 15, Germany), and the pellet was discarded to obtain the cleared cell extract. All steps were performed below 4 °C, and the extract was immediately analyzed.

**Cell Viability Assay.** The cells suspension obtained by the method mentioned above was washed by centrifugation at 1500 rpm for 5 min and resuspended in D-Hanks three times. Then the cells suspension was mixed with Muse Count & Viability Reagent (200X) and incubated at 37 °C for 5 min. Cell viability was measured using the Muse Cell Analyzer (Merck Millipore, Germany). The MTT assay was conducted to investigate the cytotoxicity of the probe. The cells (1 × 10^5 cells mL^-1) were added into the centrifuging tube with a volume of 200 μL. Then the cells were incubated with the fluorescence probes for 1 h. The concentrations of probes were the same as mentioned
above. After that, 100 μL of MTT solution (0.5 mg mL⁻¹ in PBS) was added to the tube. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to the tube to dissolve the formazan crystals. Then the cell suspension was added into a replicate 96-well microtiter plate. The absorbance was measured at 490 nm with a RT 6000 microplate reader. Cell viability was then expressed by the ratio of the absorbance of the cells incubated with the probe to that of the cells incubated with the culture medium only.

**Single-Cell Metabolomics Analysis Based on MFD-MD.** To achieve consecutive single-cell metabolomics analysis, a consecutive gated injection described in our previous article was adopted. The microchannels were rinsed sequentially with 0.1 M NaOH, ultrapure water and PBS buffer (pH6.0) for three times before flushing with electrophoresis buffer for 10 min. Then the buffer (B), buffer waste (BW), and auxiliary (A) reservoirs each were filled with 13 μL of electrophoresis running buffer, sample waste (SW) reservoir was filled with 8 μL electrophoresis running buffer, and the sample reservoir (S) was filled with 12 μL cell suspensions. After the cells were transported into the channel and loaded at separation channel as the reported method, cytolysis and electrophoresis separation were then performed immediately under a high electric field with the buffer reservoir at 2600 V, sample waste reservoir at 2600 V, sample waste reservoir at 0 V, auxiliary reservoir and sample reservoir at floating for 84 s. Finally, the multicolor fluorescent signals of H₂O₂, GSH, and Cys were simultaneously obtained by the new MFD-MD.

### RESULTS AND DISCUSSION

**Fluorescence Detection of H₂O₂, GSH, and Cys and Signal Correction.** For the fluorescent detection of H₂O₂, GSH, and Cys, H₂O₂ reacted with a probe FS to produce a fluorophore dichlorofluorescein (DCF) with a maximum emission at 525 nm, whereas GSH and Cys were individually labeled with the same probe Cy-3-NO₂, resulting in distinguishable fluorescence emission with maximum emissions at 805 and 755 nm, respectively. The reactions between the target molecules and their probes and the fluorescence spectra of the products, DCF (corresponding to H₂O₂), Cy-3-NO₂-GSH (corresponding to GSH), and Cy-3-NO₂-Cys (corresponding to Cys), are shown in Figures S1 and S2. It has been reported that the three probes had good selectivity for specific recognition of H₂O₂, GSH, and Cys. A confocal fluorescent imaging of primary liver cells incubated with aforementioned probes was performed to verify the different signals of H₂O₂, GSH, and Cys. Fluorescent images in Figure S3 show that cellular H₂O₂, GSH, and Cys could be separately represented by fluorescence with different emission wavelength. Although the images allow us to obtain information in a natural and intuitive way, absolute quantitation could not achieved by direct imaging. Moreover, spectral overlapping is a potential problem in multicolor detection. There was no spectral overlap between the green fluorescence and red fluorescence, but the red fluorescence from Cys overlapped with the near-infrared fluorescence from GSH. The emission spectrum from fluorescent dyes may appear in more than one detection channel. In the imaging, to avoid spectral interference, the signals with wavelength ranges of 700–740 nm and 760–800 nm were collected for the Cys and GSH, which deviated from the maximum fluorescence emission and caused large signal losses.

To simultaneously and quantitatively detect the fluorescent signals of H₂O₂, GSH, and Cys in single cells, a new two-laser excitation and three-channel fluorescence detection system was designed and built as described in the Experimental Section. To eliminate the errors brought by spectral overlapping, a series of methods including spectral separation, microchip electrophoresis separation, and signal correction were adopted in our multicolor detection based on MFD-MD. First, the wavelength selection of SEDBS-2 (reflection bandwidth, 650–778 nm; transmission bandwidth, 785–900 nm) and band-pass filters (BP-2, 745–765 nm and BP-3, 795–815 nm) could guarantee efficient fluorescence collection including the maximal emission of Cy-3-NO₂-Cys and Cy-3-NO₂-GSH and dramatically filter the interferential signals from each other. Second, the target signal and interferential signals from other fluorophores were effectively separated by microchip electrophoresis based on different migration times, allowing the interferential signal to be eliminated. Third, fluorescence compensation was used to correct multicolor fluorescence data for spectral overlap. It enables correction of spillover from the various fluorophores into each of the detector channels. A detailed description about the fluorescence compensation has been introduced in the previous work. As shown in Figure S4a, in our MFD-MD, H₂O₂, Cys, and GSH were detected in channel 1, channel 2, and channel 3. The electrophoretic peaks a and a’ were regarded from Cys, peaks b and b’ were regarded from GSH, and c was regarded from H₂O₂ according to their migration time. The H₂O₂ did not contribute any level of fluorescence signal to other detection channels (channel 2 and channel 3), so fluorescence compensation was not needed for H₂O₂. However, the fluorescence signals from Cys and GSH have spillover in each other channel (peak a’ in the channel 3 and peak b’ in the channel 2). The ratio of the peak area of the spillover signal of Cys (peak a’ in channel 3 to the peak area of the signal of Cys (peak a) in channel 2 was calculated to be 2.7%, and the ratio of the peak area of the spillover signal of GSH (peak b’) in channel 2 to the peak area of the signal of GSH (peak b) in channel 3 was calculated to be 1.3%. Finally, the fluorescence signal of each fluorophore was obtained by summing up all measured signal of this fluorophore in each channel (Figure S4b) and quantitative detection achieved as described below.

**Analysis Characterization.** In multiple-molecule detection, effective signal separation is crucial. High fluorescence intensity, rapid migration rate, and high column efficiency are also important in microchip electrophoresis. To obtain effective microchip electrophoresis separation and sensitive detection of H₂O₂, GSH, and Cys, the pH and concentration of the electrophoresis buffers and the separation electric field were optimized (Figure S5). Finally, 100 mM PBS buffer (pH 7.4) and a 440 V/cm separation electric field were selected as a compromise among high signal intensity, short analysis time, and improved resolution. The pH of the PBS buffer used to wash the channel was optimized to pH 6 to obtain a large peak area and a high column efficiency. Under these conditions, electrophoretic separation of DCF, Cy-3-NO₂-GSH, and Cy-3-NO₂-Cys was performed, and their three-color fluorescence signals were independently collected using the MFD-MD. Figure 2 presents typical electropherograms of the fluorescent derivatives of the standards H₂O₂, GSH, and Cys. The three completely separated peaks at the red line were identified as H₂O₂, GSH, and Cys based on their individual migration times of 71 s, 51 s, and 41 s, respectively. We also obtained good
reproducibility in determining the migration time 10 times, with RSDs of 1.4%, 1.2%, and 1.2% for H$_2$O$_2$, GSH, and Cys, respectively. For the detection of different concentrations of H$_2$O$_2$, GSH, and Cys, we observed that the signal intensities (peak areas) linearly increased with the concentrations of the target molecules in a certain range. The analytical parameters of this method, such as the linear range, limit of detection (LOD), and reproducibility were evaluated, and the data are shown in Table S1. The LOD was 0.1 nmol/L, 0.01 μmol/L, and 0.02 μmol/L for H$_2$O$_2$, GSH, and Cys, respectively. The calculated mass LOD was 11 zmol, 11 amol, and 22 amol, respectively, based on an injection volume of 110 pL.

**Simultaneous Detection of H$_2$O$_2$, GSH, and Cys at Single-Cell Level.** We next performed simultaneous detection of intracellular H$_2$O$_2$, GSH, and Cys to evaluate the applicability of the MFD-MD. Microchip electrophoresis is particularly suitable for the separation of multiple contents in a complex biological system. A primary mice liver cells suspension was analyzed using this detection system. After microchip electrophoresis and multicolor fluorescence detection, three completely separated peaks corresponding to each cell were obtained. Typical electropherograms of three individual liver cells are shown in Figure 3. The three peaks could be separately identified as H$_2$O$_2$, GSH, and Cys based on their individual migration times by Figure 2. Because of the good selectivity of the probes, nothing else showed up in the electropherograms, even in the minutes after what is shown. The calculated concentrations of H$_2$O$_2$, GSH, and Cys in single primary mice liver cells were 0.492 amol, 12.4 fmol, and 838 amol, respectively. The calculated mass LOD was 11 zmol, 11 amol, and 22 amol, respectively, based on an injection volume of 110 pL.

**Evaluation of Multiple Metabolites in Primary Mice Liver Cells.** Simultaneous detection of multiple metabolites, particularly ROS and antioxidants, in ethanol-induced oxidative stress is significant for studies of liver injury and the development of alcoholic liver disease (ALD). In this study, to assess the heterogeneity of cell damage and further investigate the molecular mechanism in ethanol-induced liver injury, H$_2$O$_2$, GSH, and Cys were simultaneously detected in single acute ethanol-treated primary mice liver cells (ethanol-treated group) using the method. In addition, silymarin, a protective agent of the liver by inhibiting oxidative stress, was chosen to treat the liver cells after ethanol induction. The concentrations of H$_2$O$_2$, and Cys and GSH in the cells treated with ethanol and then protected by silymarin (silymarin-treated group) were also evaluated by the MFD-MD. Typical electropherograms of single primary mice liver cells in the ethanol-treated group and control group (primary mice liver cells without any drug stimulation) are shown in Figure 4. The concentrations of H$_2$O$_2$, GSH, and Cys in each cell of the three groups were obtained according to the formula in Table S1. Cell-to-cell variability can be observed from a scatter diagram representing the concentrations distribution of H$_2$O$_2$, GSH, and Cys in individual 100 cells and a bar graph showing the H$_2$O$_2$, GSH, and Cys average contents with error bars in the three groups (Figure 5). The single-cell analysis result indicates the heterogeneity of the cells in ethanol-induced oxidative stress and antioxidant protection by silymarin. Moreover, we can see an average 3.69-fold increase in the intracellular H$_2$O$_2$ level and 2.47-fold and 4.08-fold decrease in the GSH and Cys levels.
Figure 5. (a) Intracellular H2O2, Cys, and GSH amounts in individual primary mice liver cells in the control group (green points), ethanol-treated group (red points), and silymarin-treated group (blue points). Each point represents one cell. The numbers of measured cells in each group are all 100. (b) Intracellular H2O2, GSH, and Cys average concentrations with error bars in the control group (a), ethanol-treated group (b), and silymarin-treated group (c). Y-axes: dark red line, red line, and green line represent the amounts of GSH, Cys, and H2O2, respectively. The numbers of measured cells in each group are all 100.

levels in the ethanol-treated group compared with those in the control group. Meanwhile, compared with those in the ethanol-treated group, H2O2 concentration in silymarin-treated group significantly decreased (2.06-fold less than that in ethanol-treated group), and the intracellular GSH and Cys levels increased (1.96-fold and 2.94-fold more than that in ethanol-treated group). This result confirms the reported antioxidant and cytoprotective properties of silymarin. Furthermore, a relationship between silymarin and Cys, that silymarin not only acts on the increase of GSH levels but also help to raise the Cys, was pointed out by our research. A cell viability assay using Count & Viability Reagent (200 µl) indicated that cell viability was maintained at greater than 90% after acute ethanol exposure of the mice and cells. An MTT assay showed that the performance of the proposed method; and viability of liver cells in control group (PDF).

■ CONCLUSIONS

In this paper, we have presented a new microfluidic detection device with two-laser excitation and three-channel fluorescence detection. The device provides a powerful tool for simultaneous single-cell analysis of H2O2, GSH, and Cys to further investigate oxidative stress in primary liver cells from acute ethanol-treated mice. This study demonstrates that (1) the microfluidic chip and electrophoresis enable accurate single-cell manipulation and effective electrophoretic separation of multiple metabolites; (2) based on the new two-laser excitation and three-channel fluorescence detection, H2O2, GSH, and Cys can be simultaneously detected with high specificity and sensitivity. It also provides a flexible strategy allowing the detection of other dyes by changing the wavelengths of the lasers and other optical elements; (3) there is significant cell diversity or cell heterogeneity among primary liver cells. The MFD-MD provides a new tool for single-cell metabolomics research, and these results provide a more accurate understanding of the intracellular oxidative/antioxidative molecular mechanism in response to external stimuli.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b01775.

Reactions of Cy-3-NO2 with Cys, GSH, and FS with H2O2; fluorescence spectra of DCF, Cy-3-NO2-Cys, and Cy-3-NO2-GSH and transmission spectra of band-pass filters and dichroic beam splitter; fluorescent images of Cys, GSH, and H2O2 in primary liver cells; fluorescent signal correction in multicolor fluorescence detection; optimization of electrophoresis conditions; analytical performance of the proposed method; and viability of liver cells in control group (PDF).

■ AUTHORS INFORMATION

Corresponding Authors
* E-mail: tangh@sdu.edu.cn.
* E-mail: liulu5252@163.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the 973 Program (Grant 2013CB933800) and the National Natural Science Foundation of China (Grants 21227005, 21390411, 21535004, and 21205074).

■ REFERENCES

(1) Avesar, J.; Arye, T. B.; Levenberg, S. Lab Chip 2014, 14, 2161− 2167.
(10) Zenobi, R. Science 2013, 342, 1243259.