Supporting Information

Simultaneous quantification of multiple endogenous biothiols in cancer cells based on a multi-signal fluorescent probe

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Tchebichef image moments

Tchebichef image moments (TMs) have the advantages of excellent description ability with the powerful multi-resolution capability and inherent invariance properties and have widely used in image processing.[1, 2] The description of TMs is shown as follows:

For an image with a size of $N \times M$, $f(x,y)$ is the image intensity function at the position of (x,y). So the image moments $T_{n,m}$ with orders of n and m can be defined as:

$$T_{n,m} = \frac{1}{\rho(n,N)\rho(m,M)} \sum_{x=0}^{N-1} \sum_{y=0}^{M-1} t_n(x) t_m(y) f(x,y)$$
$$(n = 0,1,2,\ldots,N-1, m = 0,1,2,\ldots,M-1)$$

where $\rho(n,N)$ and $\rho(m,M)$ are the normalized square-norm. $t_n(x)$ and $t_m(y)$ are the discrete Tchebichef polynomial of n and m, respectively.

$$\sum_{x=0}^{N-1} t_m(x) t_n(x) = \rho(n,N)\delta_{nm}(0 \le n \le N-1, 0 \le m \le M-1)$$

where δ_{nm} is the Kronecker symbol, $\rho(n,N)$ is the square-norm and $t_n(x)$ is the discrete Tchebichef polynomial that confirms to the recurrence relation:

$$(n+1)t_{n+1}(x) - (2n+1)(2x-N+1)t_n(x) + n(N^2-n^2)t_{n-1}(x) = 0,$$
$$n = 1,2,\ldots,N-1.$$

In order to avoid the numerical error, the normalizing Tchebichef polynomial was proposed by Mukundan et al.[3] Then the Tchebichef moments can be defined as:

$$T_{n,m} = \frac{1}{\tilde{\rho}(n,N)\tilde{\rho}(m,M)} \sum_{x=0}^{N-1} \sum_{y=0}^{M-1} \tilde{t}_n(x) \tilde{t}_m(y) f(x,y),$$
$$(n = 0,1,2,\ldots,N-1, m = 0,1,2,\ldots,M-1).$$

The following normalized recurrence formulas are employed to accelerate the computation of Tchebichef polynomials:

$$\tilde{t}_n(x) = \frac{(2n-1)(2x-N+1)\tilde{t}_{n-1}(x)}{nN} - \frac{(n-1)[N^2-(n-1)^2]\tilde{t}_{n-2}(x)}{nN^2},$$

$$\tilde{t}_0(x) = 1,$$

$$\tilde{t}_1(x) = \frac{2x+1-N}{N},$$

$$\tilde{\rho}(n) = \left(\frac{2n-1}{2n+1}\right)\left(1-\frac{n^2}{N^2}\right)\tilde{\rho}(n-1),$$

$$\tilde{\rho}(0) = N.$$

The root mean square error (*RMSE*) can be calculated using the formula：

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \tilde{y}_i)^2}{n}}$$

where n is the number of the calibrated samples, y_i and $\tilde{y}_i$ are the experimental and predicted values, respectively.

All calculation programs were written in M-file based on MATLAB 7.0 and carried out with a PC (CPU 4.00 GHz, RAM 32.0 GB). The MCR-ALS toolbox for MATLAB was downloaded from the website (http://www.mcrals.info/). N-PLS toolbox was downloaded from the website (http://www.models.life.ku.dk/algorithms).

References

1. H. L. Zhai, B. Q. Li, J. Chen, X. Wang, M. L. Xu, J. J. Liu and S. H. Lu, *TrAC-Trends Anal. Chem.*, 2018, **103**, 119-125.
2. I. Batioua, R. Benouini, K. Zenkouar, A. Zahi and E. Hakim, *Pattern Recogn.*, 2017, **71**, 264-277.
3. R. Mukundan, *IEEE. Trans. Image Process.*, 2004, **13**, 1055-1059.

The excitation spectrum of the three biothiols with a degree of partial overlap:

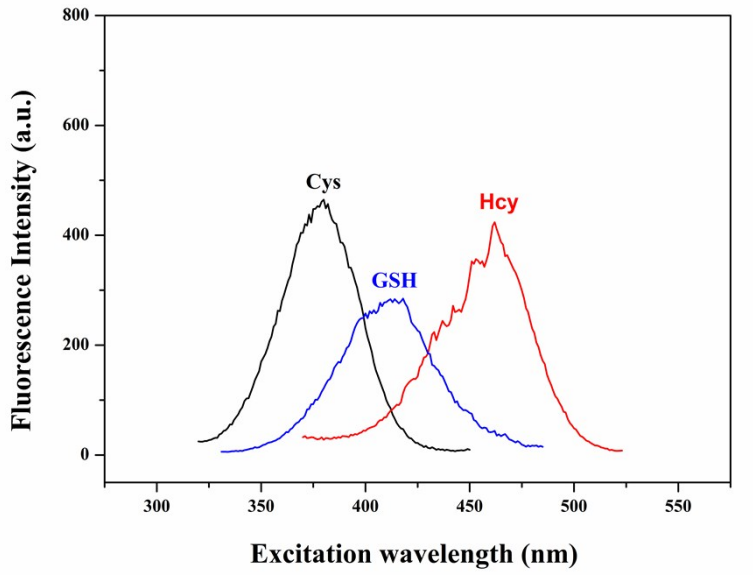

Figure S1. The excitation spectrum of the probe (10 μM) in the presence of 50 μM Cys, 50 μM GSH and 50 μM Hcy in DMSO-PBS (v/v, 6/4) at room temperature recorded after 15 min later.

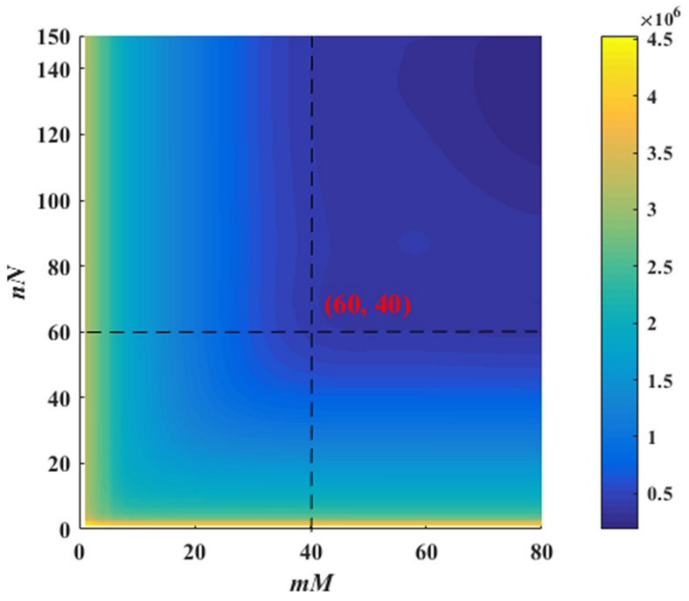

Figure S2. The approximate values of the maximum orders of *nN* and *mM* were obtained based on the reconstruction errors. (The reconstruction errors increases from the deep blue to yellow color)

Table S1. Experimental concentrations and calculated concentrations (mM) of the three thiols for different methods.

No.	Cys				GSH				Hcy			
	Exp.	Cal.			Exp.	Cal.			Exp.	Cal.		
		TM	MCR-ALS	N-PLS		TM	MCR-ALS	N-PLS		TM	MCR-ALS	N-PLS
Calibration set												
15	0.018	0.018	0.021	0.017	0.057	0.058	0.067	0.061	0.026	0.026	0.025	0.026
13	0.080	0.076	0.070	0.077	0.079	0.080	0.076	0.077	0.060	0.060	0.057	0.060
20	0.011	0.012	0.008	0.011	0.065	0.069	0.062	0.067	0.010	0.011	0.011	0.011
23	0.010	0.011	0.007	0.010	0.062	0.066	0.063	0.067	0.015	0.012	0.011	0.014
11	0.045	0.044	0.054	0.044	0.025	0.024	0.025	0.021	0.012	0.012	0.017	0.013
5	0.035	0.036	0.039	0.034	0.052	0.052	0.059	0.051	0.044	0.043	0.047	0.046
21	0.014	0.012	0.010	0.012	0.072	0.068	0.063	0.067	0.005	0.006	0.011	0.010
22	0.015	0.013	0.010	0.012	0.078	0.078	0.075	0.078	0.017	0.016	0.016	0.016
1	0.060	0.061	0.056	0.059	0.015	0.018	0.015	0.022	0.022	0.022	0.020	0.023
26	0.050	0.051	0.059	0.056	0.083	0.084	0.084	0.086	0.028	0.028	0.036	0.029
10	0.040	0.042	0.042	0.041	0.068	0.072	0.070	0.065	0.052	0.052	0.050	0.049
2	0.025	0.023	0.034	0.029	0.080	0.077	0.078	0.079	0.024	0.025	0.035	0.026
17	0.013	0.014	0.010	0.013	0.063	0.060	0.065	0.063	0.032	0.030	0.022	0.032
4	0.085	0.087	0.080	0.086	0.086	0.084	0.079	0.085	0.046	0.048	0.049	0.049
14	0.017	0.018	0.023	0.017	0.070	0.072	0.075	0.069	0.030	0.031	0.030	0.027
8	0.070	0.070	0.064	0.067	0.050	0.053	0.055	0.052	0.055	0.055	0.038	0.056
18	0.016	0.017	0.013	0.015	0.074	0.074	0.072	0.072	0.025	0.027	0.026	0.025
24	0.008	0.010	0.007	0.010	0.060	0.058	0.056	0.060	0.009	0.011	0.009	0.011
16	0.019	0.017	0.025	0.017	0.076	0.075	0.084	0.076	0.023	0.022	0.027	0.024
7	0.065	0.065	0.065	0.066	0.077	0.074	0.070	0.077	0.035	0.034	0.036	0.029
Prediction set												
19	0.020	0.018	0.018	0.019	0.045	0.047	0.048	0.049	0.020	0.020	0.013	0.019
25	0.012	0.011	0.009	0.011	0.055	0.051	0.051	0.054	0.014	0.014	0.008	0.015
3	0.055	0.052	0.052	0.052	0.084	0.088	0.084	0.080	0.050	0.054	0.054	0.049
12	0.030	0.029	0.033	0.029	0.100	0.105	0.100	0.092	0.048	0.052	0.057	0.045
9	0.090	0.092	0.083	0.090	0.035	0.033	0.031	0.040	0.040	0.038	0.036	0.041
6	0.075	0.067	0.063	0.066	0.082	0.085	0.074	0.076	0.042	0.046	0.045	0.040

Table S2. Comparison of different methods for the quantitative analysis of the three thiols.

Models	thiols	Calibration					Cross-validation		Prediction		RSD (% , n=3)		Recovery
		R	R_{adj}	*F*-test	*p*-value	*RMSE*	$R_{LOO\text{-}cv}$	$RMSE_{cv}$	R_p	$RMSE_p$	Intra	Inter	(%)
TM	Cys	0.9977	0.9973	1156	6.58E-19	0.0436	0.9963	0.0021	0.9946	0.0037	5.2	7.7	105.9
	GSH	0.9900	0.9880	259.4	8.97E-14	0.0520	0.9860	0.0029	0.9939	0.0062	5.6	8.7	97.0
	Hcy	0.9968	0.9954	341.7	1.57E-13	0.0387	0.9938	0.0017	0.9914	0.0035	4.3	6.5	97.90
MCR-ALS	Cys	0.9740	0.9726	333.0	4.64E-13	0.0768	0.9676	0.0062	0.9909	0.0062	7.7	12.1	84.62
	GSH	0.9586	0.9536	96.2	5.37E-10	0.0742	0.9460	0.0058	0.9875	0.0042	5.9	9.2	98.51
	Hcy	0.9230	0.9135	48.9	8.87E-08	0.0800	0.8975	0.0068	0.9871	0.0058	9.2	14.5	103.1
N-PLS	Cys	0.9962	0.9960	2345	1.60E-20	0.0539	0.9932	0.0029	0.9945	0.0222	4.8	7.6	87.08
	GSH	0.9877	0.9870	717.3	5.92E-16	0.0632	0.9754	0.0039	0.9977	0.0255	4.8	7.4	96.70
	Hcy	0.9909	0.9903	971.2	4.09E-17	0.0539	0.9826	0.0029	0.9964	0.0135	5.0	7.7	97.07

correlation coefficients (*R*), adjusted correlation coefficients (R_{adj}), root mean square error (*RMSE*), correlation coefficient with leave-one-out ($R_{LOO\text{-}cv}$), root mean square error of cross validation ($RMSE_{cv}$), correlation coefficients of prediction (R_p), root mean square error of prediction ($RMSE_p$).