Analysis of colonic autofluorescence spectra using multivariate curve resolution alternating least squares

Lina Liu (刘丽娜)[1], Changshun Yang (杨常顺)[2], Weihua Li (李伟华)[2], Buhong Li (李步洪)[1*], and Shusen Xie (谢树森)[1]**

[1]*Key Laboratory of OptoElectronic Science and Technology for Medicine of Ministry of Education, Fujian Provincial Key Laboratory for Photonics Technology, Fujian Normal University, Fuzhou 350007, China*

[2]*Department of Surgical Oncology, Fujian Provincial Hospital, Fuzhou 350001, China*

[*]*Corresponding author: bhli@fjnu.edu.cn;* [**]*corresponding author: ssxie@fjnu.edu.cn*

Received January 27, 2014; accepted March 27, 2014; posted online April 30, 2014

Autofluorescence (AF) spectra of colonic normal and adenocarcinoma tissues are measured under excitation of 337 nm and analyzed by multivariate curve resolution alternating least squares (MCR-ALS) method using non-negativity constraint. Collagen, nicotinamide adenine dinucleotide hydrate (NADH) and elastin are identified as the main contributing biomedical components. Fisher's discrminant anlysis (FDA) on the concentration profiles of the principle components (PCs) shows acceptable sensitivity, specificty and accuracy for discrminanting the adenocarcinoma tissues from the normal tissues. MCR-ALS is a powerful tool for characterzing the spectra profiles of the main biochemical components in neoplasm transformation.

OCIS codes: 170.6510, 300.2530.

doi: 10.3788/COL201412.051705.

Autofluorescence (AF) spectroscopy is a useful tool for early detection of cancer[1−3]. The metabolic and structural alterations during tissue neoplastic transformation could be reflected in changes in the AF profile of the endogenous fluorophores[2−4]. However, fluorescence spectra recorded from tissue can be distorted and thereby limiting the accuracy of the extracted information[5]. To resolve such limitations, one strategy is to use the intrinsic property of multi-modal measurement such as diffuse reflectance spectroscopy or Raman spectroscopy combined with fluorescence spectroscopy to suppress the effects of scattering and absorption or extract more information[5−7]. Another strategy is to use mathematical resolution method to recover the spectra profiles of the corresponding components from the distorted multivariate data[8,9]. Multivariate curve resolution alternating least squares (MCR-ALS) method generates spectral components to minimize the fitting error for a given spectroscopy by applying natural constraints during alternating least-squares optimization. It has shown an improving resolution by modeling rather than discarding the effect of unexpected components[10] and this approach has been used to extract the biochemical principle components (PCs)[8,9]. In this letter, AF spectra of colonic tissues are analyzed using MCR-ALS with non-negativity constraint. Fisher's discriminant analysis (FDA) is applied on the concentration profiles of PCs to find differences lying in the spectra of adenocarcinoma and normal tissues.

A total of 72 colon tissue specimens (38 normal and 34 adenocarcinoma) were obtained from 25 patients (15 males and 10 females, 50-70 years old, median age 58 years) during surgery. This study was approved by the Research Committee of Fujian Provincial Hospital. The specimens were rinsed with saline immediately after resection and fluorescence examination was carried out within 2 h. After spectra acquisition, specimens were fixed in 4% formalin and subjected for histological examination.

AF spectra were measured on a spectrofluorometer (FLS920, Edinburgh Instruments, UK) under 337 nm excitation[2]. Specimen was mounted on a tilted quartz slide and excited at 60° incident angle and the fluorescence emission (360 to 650 nm) was collected twice[11]. Although the best excitation wavelength for collagen and nicotinamide adenine dinucleotide hydrate (NADH) in colonic tissue is 340 nm, in order to be comparable with the previous studies of other groups[12,13], 337 nm was chosen for this study. The fluorescence spectroscopy of pure NADH was measured when dissolved in sodium hydroxide while those of the insolvable pure collagen I and elastin were measured in a solid fibrous form[14].

AF spectra were smoothed first and then analyzed by MCR-ALS. More specifically, a bilinear decomposition of the measured spectral data matrix was based on the following model[10]:

$$D_{(i\times j)} = C_{(i\times k)} S^{\mathrm{T}}_{(k\times j)} + E_{(i\times j)}, \tag{1}$$

where $D_{(i\times j)}$ contains the measured spectra of each specimens in rows; $S^{\mathrm{T}}_{(k\times j)}$ represents the pure component spectra of fluorophores known to be presented in colonic tissue; $C_{(i\times k)}$ is associated with the concentration profiles of fluorophores; $E_{(i\times j)}$ is the matrix of residuals not explained by the model and close to the experimental error. After estimating the number of PCs and the pure component spectral profiles, MCR-ALS procedure was initiated with non-negativity constraint applied to the concentration and spectrum profiles[15]. The percent of lack of fit and the percent of variance explained were calculated to reveal whether experimental data were well fitted. The concentration profiles of PCs which related to the characteristics of each specimen were calculated according to

$$C = DS(S^{\mathrm{T}}S)^{-1}. \tag{2}$$

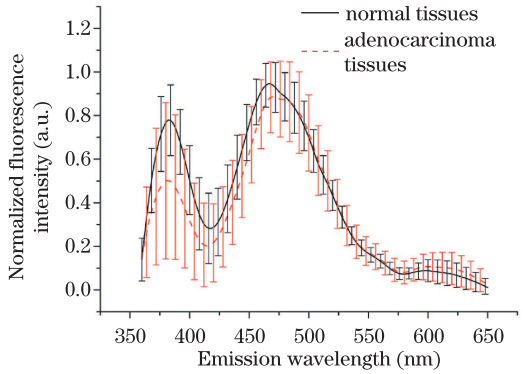

Fig. 1. (Color online) Normalized mean spectra of normal and adenocarcinoma tissues urder 337-nm excitation.

The algorithm of FDA based on leave-one-out of cross validation was described in our previous study[11]. Briefly, the k-dimensional concentrations C were projected onto a projection direction to obtain a one dimensional discriminant score. Tissue specimen was classified by comparing its discriminant score with the dividing point on the projection direction.

Figure 1 shows the normalized mean spectra of human normal and adenocarcinoma colon tissues under 337-nm excitation. Both tissues showed two major fluorescence bands centered at 380 and 470 nm, respectively. However, for 380-nm emission wavelength the fluorescence intensity of normal tissue was higher than that of adenocarcinoma. These differences indicated the intrinsic fluorophore compositions in tissue change during tissue neoplasm. Larger variations were observed in the spectra of adenocarcinoma tissues might due to the differences between the collagen fiber irregularity and the biochemical characteristics such as PH value of adenocarcinoma tissues, which could increase the variance of the discriminant parameters of adenocarcinoma tissues and in turn lower the discriminant accuracy.

To elucidate these changes, the spectra of PCs and their relative contributions to the entire AF spectra were extracted from the measured spectra using the MCR-ALS method. It was found that the first three PCs with variance greater than 1 accounted for 99.64% of the total variance, therefore only three main components needed to be extracted. With the estimation of PCs numbers and non-negative constraint, MCR-ALS was achieved with good quality since the percent of lack of fit at the optimum was 0.049% and the percent of variance explained was 99.96%, respectively. The spectra profiles of the first three PCs are shown in Fig. 2(a). In order to compare their spectral profiles with those of pure fluorophores, all of the spectra of the PCs and those of pure fluorophores were normalized to the maximum intensity value of the each spectrum individually (Fig. 2(b)). As shown in Fig. 2, the spectra of the PCs are basically comparable to the corresponding measured spectra of the pure fluorophores, which indicating that the first PCs was contributed by elastin, the second by NADH and the third by collagen, respectively. Compared the emission peak of pure collagen I with that of the MCR-ALS extracted fluorescence spectrum of collage in tissue, i.e., the third PCs, spectral shift from 390 to 380 nm and the width narrower were noticed. Banerjee *et al.*[4] suggested that the observed emission peak at 385 nm was mainly contributed by collagen VI since its FWHM was narrow. The emission peaks originating from different type of collagens might be the main reason for the spectral profile differences. Spectral shift was observed from 460 to 470 nm when compared the emission peak of pure NADH in solution with that of the MCR-ALS extracted fluorescence spectrum of NADH in tissue, which was consistent to the emission peak of the measured entire tissue fluorescence spectra as shown in Fig. 1. This spectral shift might mainly due to the different measurement environment. The differences observed between these two groups of spectra were also affected by the tissue scattering and absorption. Pure elastin has been observed to have an emission spectrum at 400−450 nm under excitation of 340−370 nm, the MCR-ALS extracted fluorescence spectrum of elastin in tissue, that is, the first PCs, exhibited two peaks profile might result from greater tissue abortion at 425 nm.

The concentrations profiles of the PCs could be regarded as the relative contributions of collagen, NADH and elastin. The ratios of the relative concentrations of PCs were shown in Fig. 3. The ratios of the relative concentrations of the first PCs divided by that of the second PCs, i.e., 1st PCs vs 2nd PCs were shown in Fig. 3(a), the mean ratio of adenocarcinoma tissues (0.22±0.12) was lower than that of normal ones (0.54±0.10) (p <0.01). Assuming that the normal tissues were identified as those with the ratio of these two PCs >0.1, it was possible to discriminate the tissue specimens with the sensitivity of 86.8%, specificity of 70.6%,

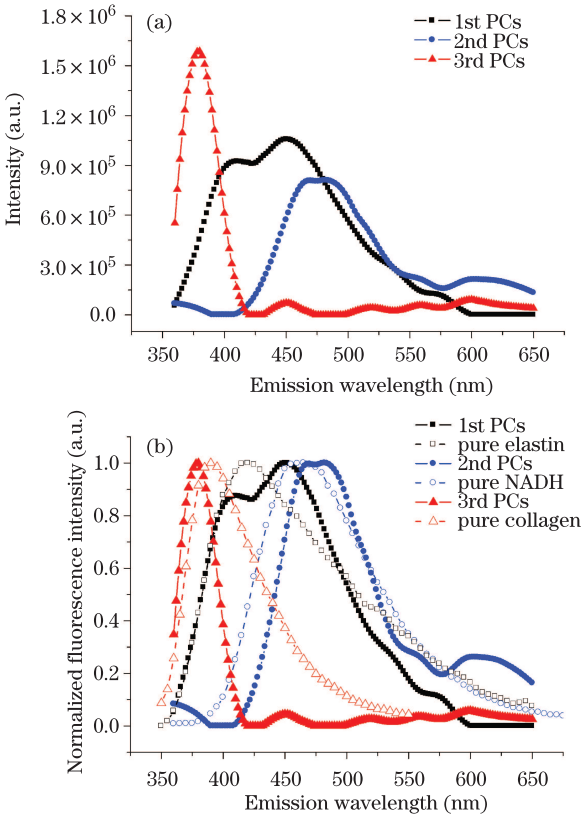

Fig. 2. (Color online) (a) Spectra of three PCs extracted from the measured data using the MCR-ALS and (b) the emission spectra of pure elastin, NADH, and collagen.

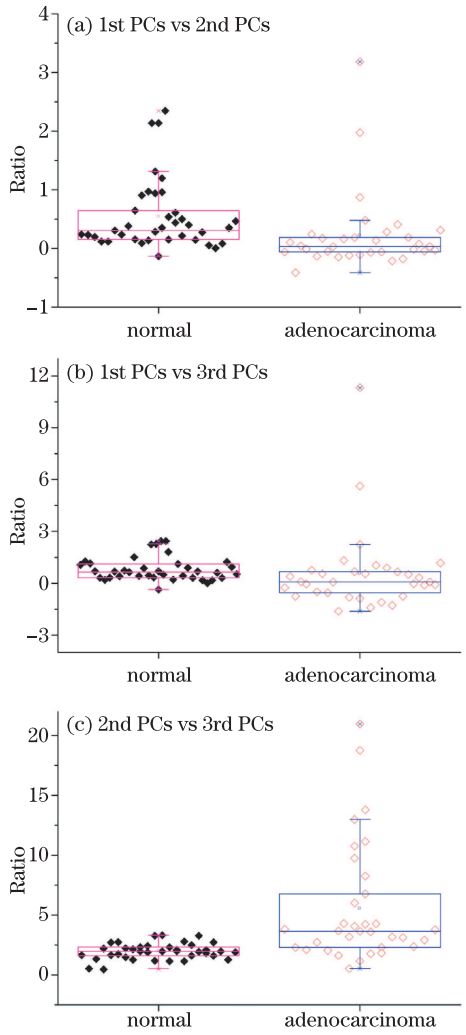

Fig. 3. (Color online) Ratios of the relative concentrations of the PCs.

and accuracy of 79.2%, respectively. For the ratios of 1st PCs vs 3rd PCs (Fig. 3(b)), there were no statistically differences observed between the ratio values of normal tissues and those of adenocarcinoma tissues. For the ratios of 2nd PCs vs 3rd PCs (Fig. 3(c)), it was found that the differences between the ratios of adenocarcinoma tissues were much greater than those of normal tissues. Meanwhile, the mean ratio of adenocarcinoma tissues (5.52±4.98) was higher than that of normal ones (1.96±0.68) (p <0.01). Assuming that the normal tissues were identified as those with the ratio of these two PCs <2.25, it was possible to discriminate the specimens with the sensitivity of 79.4%, specificity 76.3% and accuracy 77.8%, respectively.

The comparison between the ratios of 2nd PCs vs 3rd PCs concentrations and those of emission peaks values (I_{470}/I_{380}) was approximate and had the same variation trend (Figs. 4(a) and (b)), which confirmed that 380- and 470-nm emission peaks were the characteristic peaks of collagen and NADH, respectively. Linear correlation analysis of these two ratios was applied to illustrate the same variation trend (Figs. 4(c) and (d)). With the four circled offline cases removed, the goodness of fit of linear correlation (R^2=0.8974) is shown in Fig. 4(d). In addition, the ratio of peak intensities increasing from normal to adenocarcinoma tissues could originate from the relative concentrations of collagen and NADH change during tissue neoplasm. Schomacker *et al.*[12] reported that all the fluorescence spectra of colonic tissues had peaks at 390 and 460 nm which arose from collagen and NADH respectively and fluorescence intensity at 390 nm decreased from normal tissue to adenocarcinoma one. Eker *et al.*[13] reported that under 337-nm excitation only one single emission peak in the AF spectrum was seen for normal tissue (390 nm) and adenocarcinoma (close to 460 nm), which were consistent with our results that suggested that the relative concentrations of collagen and NADH indeed changed during neoplasm.

Applying FDA on the concentration profiles of the PCs, the sensitivity, specificity, and accuracy for discriminating normal from adenocarcinoma tissues in the present study were 82.4%, 84.2%, and 83.3%, respectively, which were higher than those determined using the relative concentrations of the PCs as diagnostic indicator since the contribution of elastin was included for classification. These values are comparable to those determined by applying principle component analysis-fisher's discriminant analysis (PCA-FDA) methods on the scores of the PCs from PCA[11]. In contrast to PCA, MCR-ALS uses natural constraints to break the rotational ambiguities to ensure matrix decomposition is unique and can be used for the recovery of responses of every component in the AF spectra measurement although the disturbances from biological microenvironments, absorption, and scattering are unclear.

In conclusion, this study suggests that MCR-ALS can be used as a powerful tool for characterizing the spectral profiles of the main (fluorescent) biochemical components and their changes during tissue neoplasm from the AF spectra by modeling the effect of unexpected disturbances. Three PCs corresponding to collagen, NADH, and elastin are found contributing to the AF spectra of normal and adenocarcinoma tissues. Applying FDA on the concentration profiles of the PCs, the sensitivity, specificity, and accuracy for discriminating the adenocarcinoma tissues from the normal tissues are 82.4%, 84.2%, and 83.3%, respectively.

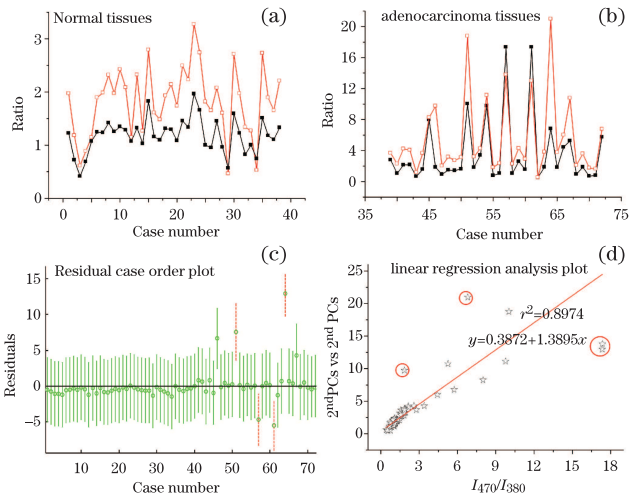

Fig. 4. (Color online) Comparision and liner regression analysis between the ratios of 2nd PCs vs 3rd PCs (circle) and those of emission peak values (square).

This work was supported by the Fujian Provincial Science and Technology Foundation (No. 2009Y0015), the Program for New Century Excellent Talents in University of China (No. NCET-10-0012), and the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT1115).

References

1. D. Fielding, J. Agnew, D. Wright, and R. Hodge, Photodiagn. Photodyn. Ther. **3,** 259 (2006).
2. B. Li and S. Xie, World. J. Gastroenterol. **11,** 3931 (2005).
3. B. Li., Z. Zhang, and S. Xie, Chin. Opt. Lett. **4,** 348 (2006).
4. B. Banerjee, B. Miedema, and H. R. Chandrasekhar, Am. J. Med. Sci. **316,** 220 (1998).
5. Z. Volynskaya, A. S. Haka, K. L. Bechtel, M. Fitzmaurice, R. Shenk, N. Wang, J. Nazemi, R. R. Dasari, and M. S. Feld, J. Biomed. Opt. **13,** 024012 (2008).
6. X. Li, J. Lin, J. Ding, D. Wang, Y. Wang, and X. Li, Chin. Opt. Lett. **3,** S153 (2005).
7. H. Liu, H. Gisquet, W. Blondel, and F. Guillemin, Biomed. Opt. express. **3,** 3278 (2012).
8. Y. Pu, W. Wang, G. Tang, and R. R. Alfano, J. Biomed. Opt. **15,** 047008 (2010).
9. Y. Pu, W. Wang, Y. Yang, and R. R. Alfano, Appl. Opt. **52,** 1293 (2013).
10. R. Tauler, A. Smilde, and B. Kowalski, J. Chemom. **9,** 31 (1995).
11. L. Liu, Y. Nie, L. Lin, W. Li, Z. Huang, S. Xie, and B. Li, Photodiagn. Photodyn. Ther. **10,** 111 (2013).
12. K. T. Schomacker, J. K. Frisoli, C. C. Compton, T. J. Flotte, J. M. Rithter, N. S. Nishioka, and T. F. Deutsch, Lasers. Surg. Med. **12,** 63 (1992).
13. C. Eker, S. Montan, E. Jaramillo, K. Koizumi, C. Rubio, S. Andersson-Engels, K. Svanberg, S. Svanberg, and P. Slezak, Gut **44,** 511 (1999).
14. R. S. DaCosta, H. Andersson, and B. C. Wilson, Photochem. Photobiol. **78,** 384 (2003).
15. T. Azzouz and R. Tauler, Talanta **74,** 1201 (2008).