A calibration-free application of near infrared spectroscopy to the determination of viable cell density in mammalian cells

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Learning with Purpose

Introduction

Near infrared spectroscopy (NIRS) is one of a number of potential in situ process analysis technologies (PATs) for application in cell culture processes. NIRS is simple to operate and well suited for the determination of the major components in cell culture, but their application requires significant calibration be made about the relationship with the NIR spectra. In this work a novel and simple calibration-free method for determination the viable cell density is proposed. Multivariate curve resolution alternating least squares (MCR-ALS) is able to 1) extract from a complex spectral feature the number of involved components, 2) attribute the resulting spectra to chemical compounds or structure responses, and 3) quantify the individual spectral contributions with or without a priori knowledge. We have evaluated MCR-ALS for the routine analysis of viable cell density in mammalian cells. And the results were compared with those obtained from the multivariate technique PLS, LS-SVM, to evaluate the results of MCR-ALS.

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Methods

Cell culture

- Cells will be grown in 125 ml flask with working volume of 30 ml in an incubator.
- Those cell samples will be analyzed using Cedex HiRes to measure cells' physiological parameters, such as VCD, TCD, Viability.

Near infrared acquisition

- The cell culture batches were monitored using a Fourier transform near infrared (FT-NIR). The spectral range is from 4000 to 10,000 cm−1.
- Each cell was measured independently for three times. The chemometric models were developed by computing the average of 3 spectra for each reference value.

Data sets and Multivariate Analysis:

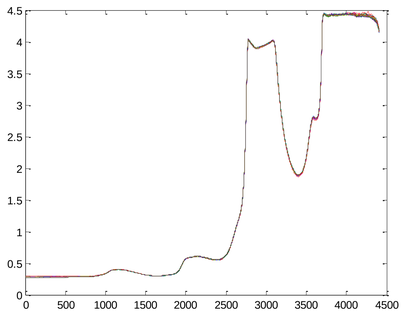
- A first data set containing different standard viable cells and dead cells was prepared (n=21, with the viability from 0~100%), which is used for calibration.
- The second data set was prepared from real cell samples (n=157), which is used for validation.
- NIR Spectra were exported and preprocessed with Unscrambler® version 10.1 (32-bit) (CAMO®Software AS, Oslo, Norway).
- For MCR-ALS, the spectra were exported to the software MCR-ALS Toolbox in Matlab (R2010b).

Theory

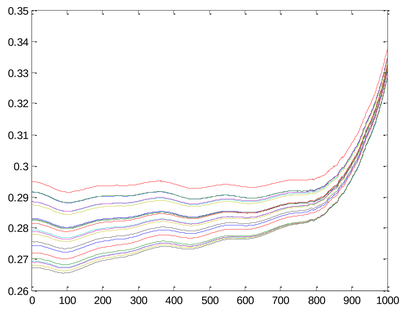
- Resolution methods allow the decomposition of the initial data matrix **D** (r ×c) into the product of two data matrices **C** (r ×n) and $\mathbf{S}^T$ (n ×c), each of them including the pure response profiles associated with the row and column direction of the initial data matrix solving the Equation represented in the following.
- $\mathbf{D} = \mathbf{C}\mathbf{S}^T + \mathbf{E}$

Results

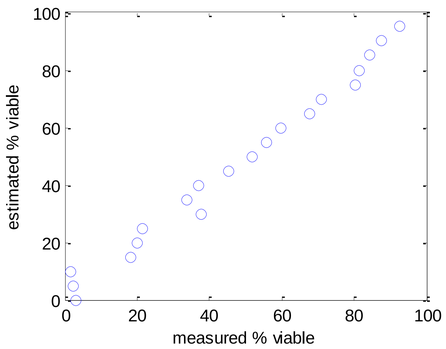
Original spectra



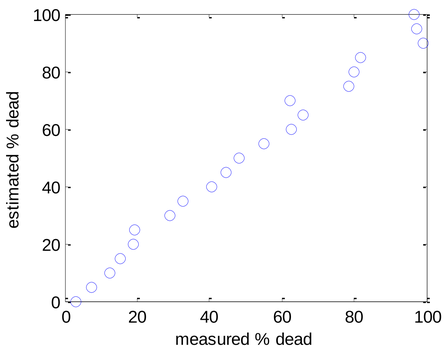
Selected variables



Viable cells

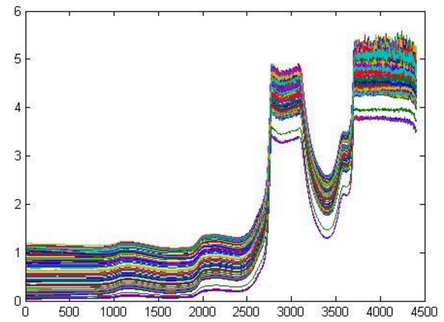


Dead cells



Data Set1

Data Set 2



	RMSEP	RSQ
PLS	1.365	0.961
LS-SVM	0.805	0.986
MCR-ALS	1.427	0.800

Conclusions

- This study has demonstrated that it is successful to quantify the viable cell density with a high degree of accuracy by using the NIR technique. The use of NIR for monitoring large scale cell culture processes for manufacturing of biopharmaceuticals is possible.
- It is potential to use this calibration-free method MCR-ALS to estimate the viable cell density.
- The problem is also shown compared to traditional multivariate calibration approaches such as PLS and LS-SVM. In the future work, we need to extract more exact information from many different cell lines towards the pure feature of viable cells and dead cells.

References

- T. Azzouz, R. Tauler. Application of multivariate curve resolution alternating least squares (MCR-ALS) to the quantitative analysis of pharmaceutical and agricultural samples. Talanta, 2008, 74: 1201-1210