Calibration by QCs

Manual

2013-05-24

1. Introduction

Various approaches have been used to reduce systematic non-biological error which is frequently occurs within and between batches in metabolomics data sets. Direct scaling, using the total signal intensity, labeled internal standards for every analyte, or a representative Internal Standard, tends to suppress the sensitivity of the analyses and thus may lead to a loss of information. Reducing systematic nonbiological error by periodically analyzing pooled samples (PQCs) along with the subject samples is gaining acceptance as a quality control strategy in metabolic profiling. Despite their success, PQC-based calibration methods have a limited capacity to adjust batch and injection order effects lacking the pretreatment of the PQC data beforehand. Additionally, the calibration process is laborious and prone to human error. Therefore, we developed a seven-step workflow and software to calibrate metabolomics data sets by pooled samples as quality controls (PQCs). The software can adjust both within- and between-batch variance and perform rough, medium, and precise calibrations. Calibrated data sets derived from various platforms can be combined directly. It is user friendly with an integrated GUI, has lots of options, and is independent to data sources.

2. Operation procedure

1) Startup

In the “current directory” panel of Matlab environment (7.1.0246+SP3) , right click the file “calibration.m” and select run (Fig. 1). The main window will appear (Fig. 2).

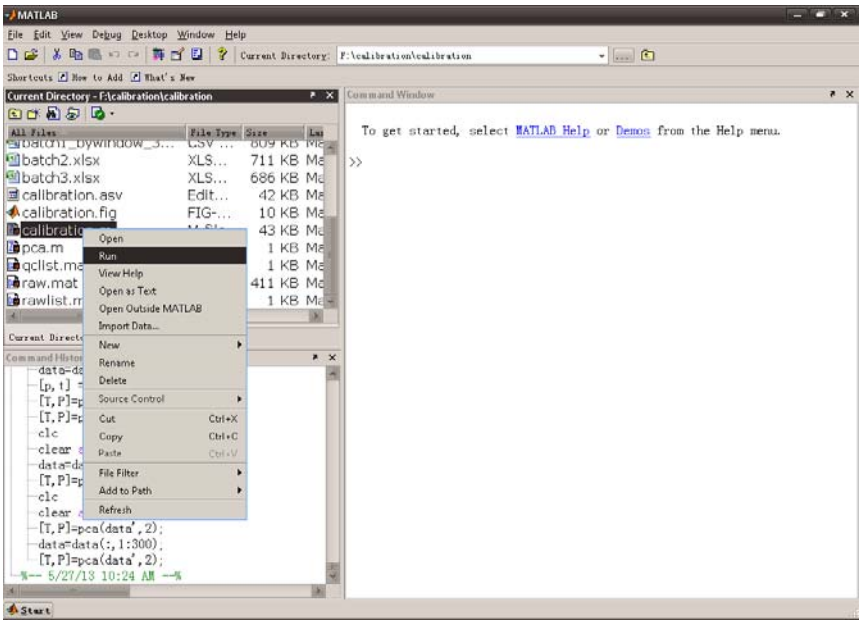

Fig1. Software startup

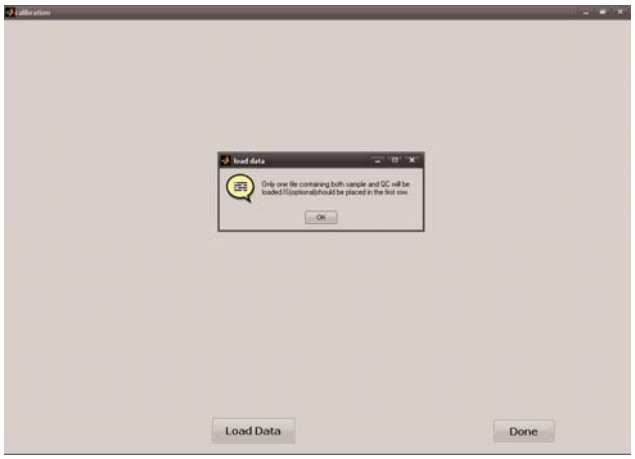

Fig. 2 The initial window

2) Import dataset

Dataset with both samples and QCs (quality controls) is required. QCs should be pooled samples. They contain the same compounds as the subject samples and are supposed to reflect the average metabolite concentrations within a study. QCs are pretreated according to the same protocols as the subject samples and are evenly injected throughout the analyses. The performances of the pretreatment and the

analytical platform can be assessed using the QCs.

Click the button “load data” to select a file for processing. Acceptable file formats are .xls, .xlsx, and .csv.

Each row is a variable (metabolite/peak) and each column is a sample or QC. Rank all the samples and QCs respectively, as instrument detection order. Samples at first. The first row and first column are supposed to be names of variables or samples (Fig. 3).

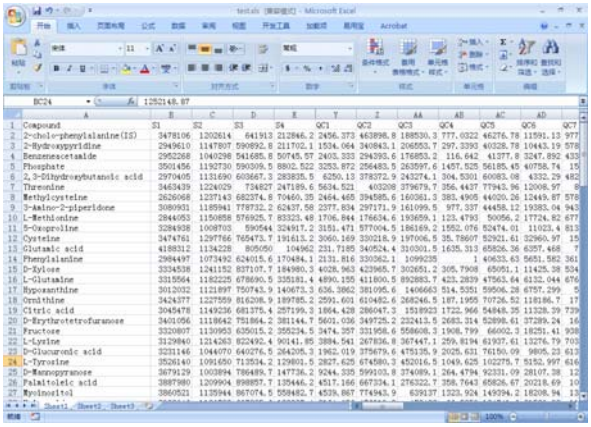

Fig. 3 format of dataset

3) Set up parameters

In the “Parameter Setting” area, change the parameters manually or as it is (Fig.4). Click “OK/Initialization” to continue.

Number of QCs: number of QCs

Number of Samples: number of samples

QC average: QC average mode (batch or window)

Batch/Window Size: size of QC average

Delete ratio (%) of RSD (QC): an RSD threshold. Variables with RSD>threshold will be removed.

Delete ratio (%) of zero (QC): a threshold for percentage of zero values. Variables with zero values>threshold will be removed.

IS Calibration: calibrate by IS

QC Calibration: calibrate by QCs

Z-Score: Z-Score transformation

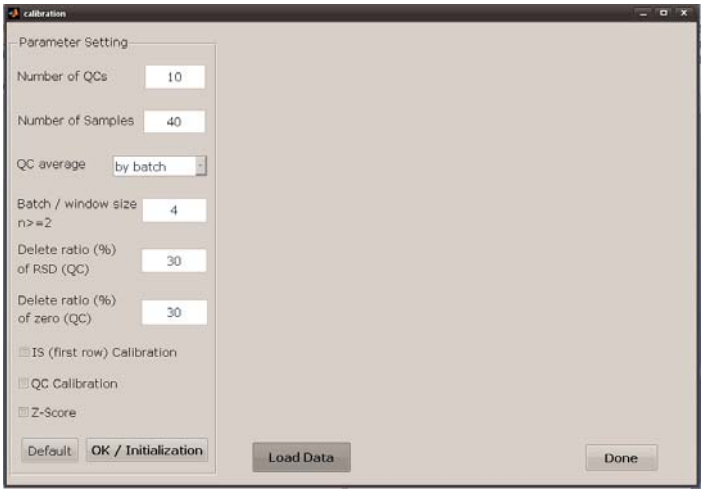

Fig. 4 set up parameters

4) QC outlier selection

After parameters setting, the “QC Outlier Selection” panel will be visiable (Fig. 5). Select and exclude QC outliers manually from the “QC List” with the help of various PCA scores plots. The plots will be saved as figures(.tiff, .ai, .fig) in specified folders. “Auto Selection” mode is recommanded which will exclude QCs deviated from 3*SD in the PCA scores plot derived from all QCs (Fig. 6). Click “OK” and continue.

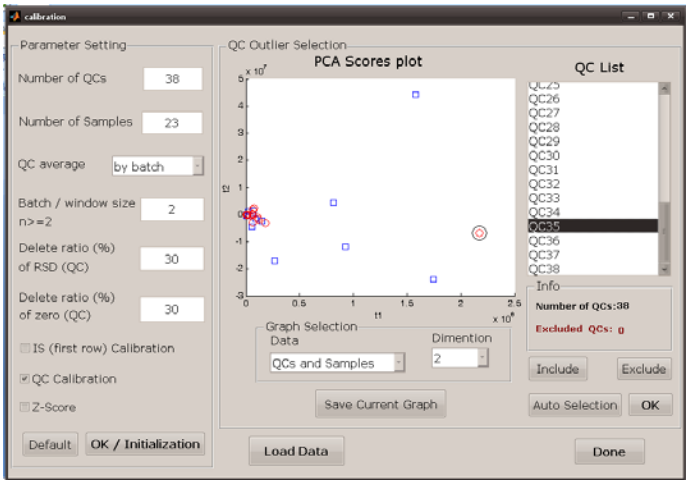

Fig. 5 QC outlier selection panel

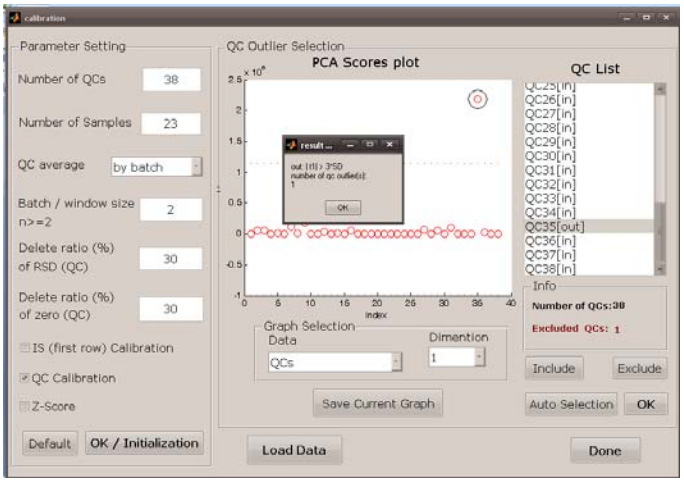

Fig. 6 Autoselection of QC outliers (QC35)

5) Calibrate the data

Click “Run and save result” and the software will calibrate the data as the parameters (Fig. 7). The workflow is shown as Fig. 8.

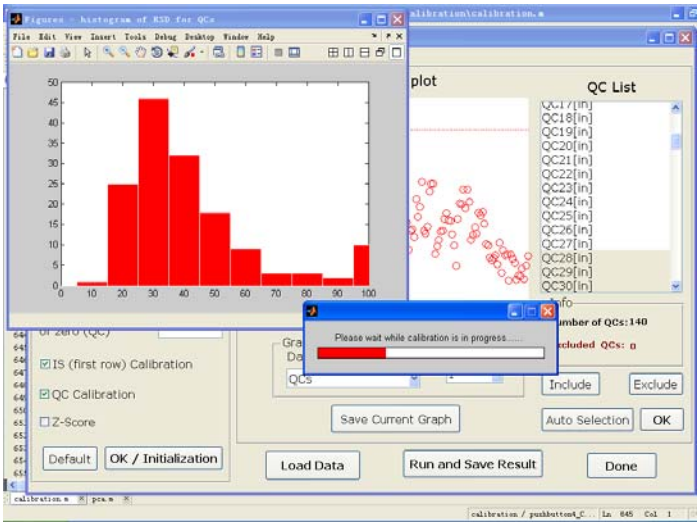

Fig. 7 Calibration in progress

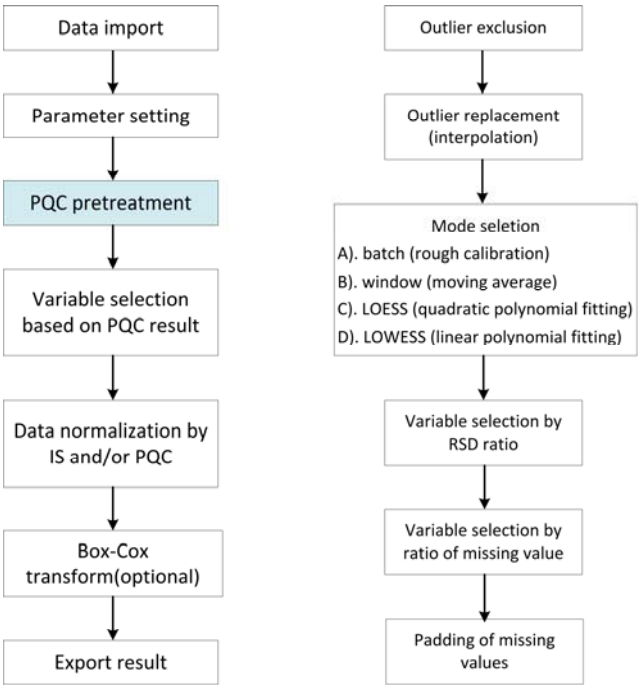

Fig. 8 workflow of calibration

6) Result output

The dataset after calibration (a .xls or.csv file) will be saved specified folder. Some relevant information will be displayed (Fig. 9).

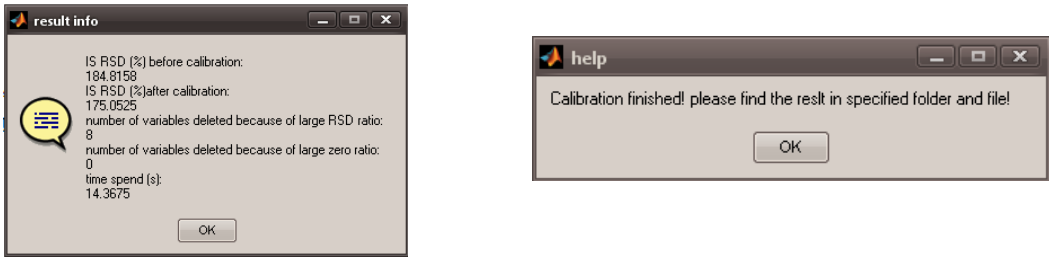

Fig. 9 Relevant information of the calibration

7) Re-calibration

Click “Done” to close the window. Or, change the parameters and click “Run and save result” button to calibrate the dataset once again.