RootProf

TUTORIAL 6

Analysis of XAS spectra

Contents

Chapter 1

The data set

Unidimensional spectra from X-ray absorption spectroscopy measurements taken in fluorescence mode compose our dataset. All spectra have been acquire by the same synchrotron beamline, in a flow cell. Experimental samples consists in pellets formed by biomasses of R. Sphaeroides grown in presence of Cr(VI). Measurements have been taken at different time, so that the kinetic of the chromate reduction from Cr(VI) to Cr(III) by bacteria can be monitored. The corresponding files are included as demo files. They are formed by two columns, the first containing the photon energy values, the second the corresponding values of photon intensity.

Table 1: Samples used for XAS analysis.

Nsample	Name	Description
0	Kinetic_01	Measurement after 40 min
1	Kinetic_02	Measurement after 55 min
2	Kinetic_03	Measurement after 80 min
3	Kinetic04	Measurement after 170 min
4	Cr3_2_exafs_s02_norm	Pure Cr(III) sample
5	Cr6_exafs_2_flow_scal02_norm	Pure Cr(VI) sample

Chapter 2

First sight analysis of XAS spectra

Motivation

Obtaining a quick and joint view of all input spectra, comparing and inspecting their features, and testing the effect of the rebinning procedure.

The command file

The list of commands is the following.

```
  whichanalysis 0
  figpaper 1
  varbin 1
! varbin 0
  range 5000 6500
  file  kinetic_01.extract
  file  kinetic_02.extract
  file  kinetic_03.extract
  file  kinetic04.extract
  file  cr3_2_exafs_s02_norm.nor
  purephase
  file  Cr6_exafs_2_flow_scan02_norm.nor
  purephase
```

The commands have been included in the demo file named *fileInputXASFirstSight*. See the user guide for an explanation of their meaning.

Running RootProf

Start ROOT by clicking on his icon, or by typing “root” on a terminal window. Then write the root command:

```
Root> .x RootProf_v15.C(“fileInputXASFirstSight”)
```

or

```
Root> .> outputXASFirstSight
.x RootProf_v15.C(“fileInputXASFirstSight”)
.>
```

After some seconds, graphic windows will start appearing on your screen, while text output will appear on the terminal window, or redirected in the file named *outputXASFirstSight*. When the run ends, the root prompt will appear again on the ROOT terminal, and you will be able to edit each single graphic window and read the output file by your text editor.

The graphic output

The graphic output of the First Sight procedure (Fig. 1) shows that the XAS spectra have different ranges and different binning. Moreover, each spectrum has a variable binning throughout its own range. The command varbin 1 forces the program to treat the spectra with variable binning. As a consequence, the data matrix plot is not produced.

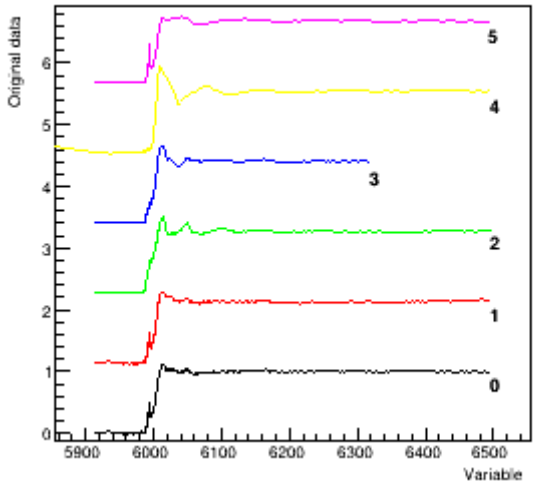


Fig.1 Original data shifted (before preprocessing)

If instead the command *varbin* 1 is commented, or *varbin* 0 is included, then the spectra are interpolated and rebinned, so that they have the same range and binning among each other and each spectrum has uniform binning. The new plot is reported in Fig.2. In these conditions the data matrix can be produced (Fig.3).

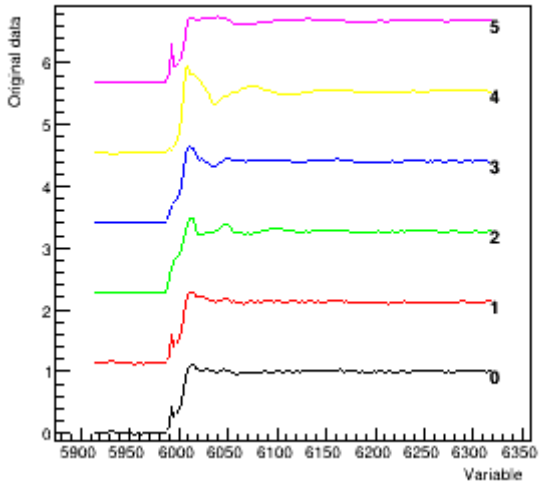


Fig.2 Original data shifted (before preprocessing)

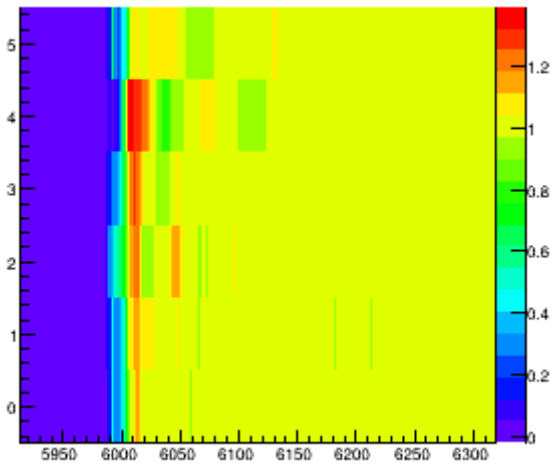


Fig.3 Data Matrix (before preprocessing)

The output file

The content of the output file named *outputXASFirstSight* is reported below, with comments added.

```
 Input from file: fileInputXASFirstSight
-------------------------------
  whichanalysis 0

  figpaper 1

! varbin 1

  range 5000 6500

  file  kinetic_01.extract

  file  kinetic_02.extract

  file  kinetic_03.extract

  file  kinetic04.extract

  file  cr3_2_exafs_s02_norm.nor

  purephase

  file  Cr6_exafs_2_flow_scan02_norm.nor

  purephase
```

The above section shows the commands read from the command file. It should be checked to ensure that they are interpreted correctly.

```
 Reading input files:
 ----------------------
Sample 0 -> file kinetic_01.extract
```

```
         Found 340  points
Sample 1 -> file kinetic_02.extract
         Found 340  points
Sample 2 -> file kinetic_03.extract
         Found 340  points
Sample 3 -> file kinetic04.extract
         Found 295  points
Sample 4 -> file cr3_2_exafs_s02_norm.nor
         Found 178  points
Sample 5 -> file Cr6_exafs_2_flow_scan02_norm.nor
         Found 170  points
```

The above section reports the number of data points read within each input file.

```
 Spectra have different binning
```

The program realizes that spectra have different binning. If the *varbin* 1 command is given, no special procedure is undertaken.

```
Spectra interpolated to have equal binning
```

```
 Transforming input spectra to equal binning
Chosen range: [5914.76 6317.77]
Number of points: 171
```

If instead *varbin* 0 or no *varbin* command is given (as in this case), the procedure to interpolate the spectra to have equal binning is activated. In the section above the equalized range and points can be read.

Chapter 3

Qualitative analysis of XAS spectra

Motivation

Apply PCA for classification of spectra having variable binning and different binning and ranges among each other.

The command file

The list of commands is the following.

```
whichanalysis 1
figpaper 1
threshold 0.96
range 5000 6500
file  kinetic_01.extract
file  kinetic_02.extract
file  kinetic_03.extract
file  kinetic04.extract
file  cr3_2_exafs_s02_norm.nor
file  Cr6_exafs_2_flow_scan02_norm.nor
```

The commands have been included in the demo file named *fileInputXASQualitative*. See the user guide for an explanation of their meaning.

Running RootProf

Start ROOT by clicking on his icon, or by typing “root” on a terminal window. Then write the root command:

Root> .x RootProf_v15.C(“fileInputXASQualitative”)

or

Root> .> outputXASQualitative

.x RootProf_v15.C(“fileInputXASQualitative”)

.>

After some seconds, graphic windows will start appearing on your screen, while text output will appear on the terminal window, or redirected in the file named *outputXASQualitative*. When the run ends, the root prompt will appear again on the ROOT terminal, and you will be able to edit each single graphic window and read the output file by your text editor.

The graphic output

PCA analysis has been applied to the spectra and data matrix shown respectively in Figs.2 and 3 of chapter 2. In Fig.1 is reported the Scree plot, and in Figs. 2, 3 respectively the loadings and scores for the first two principal components. PC1 is able to distinguish Cr(III) from Cr(IV) spectra.

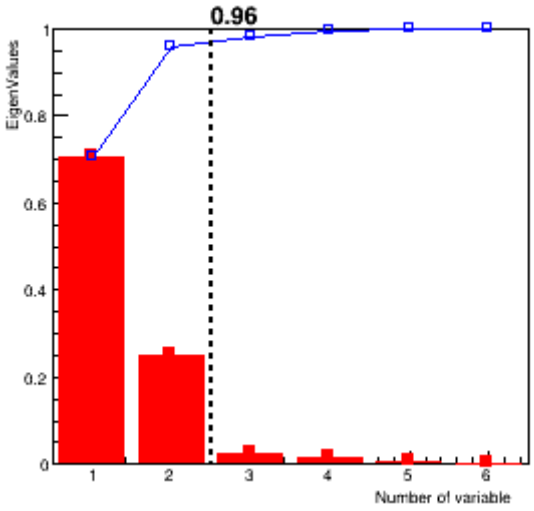


Fig.1 Scree plot

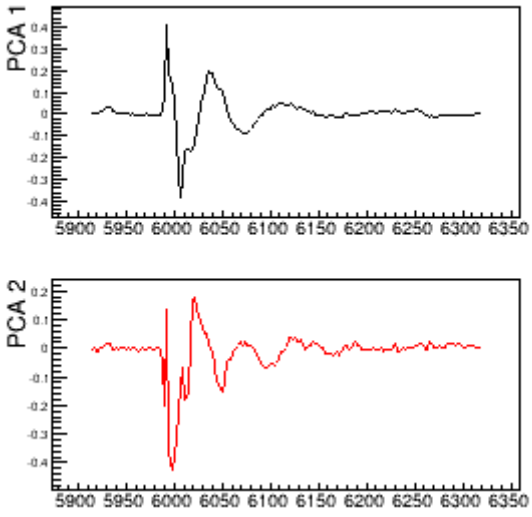


Fig.2 Loadings

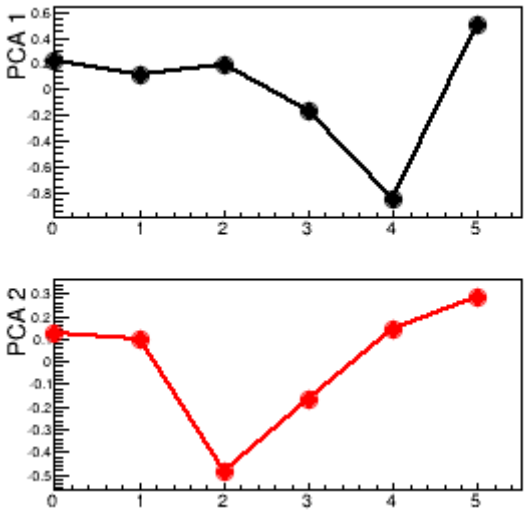
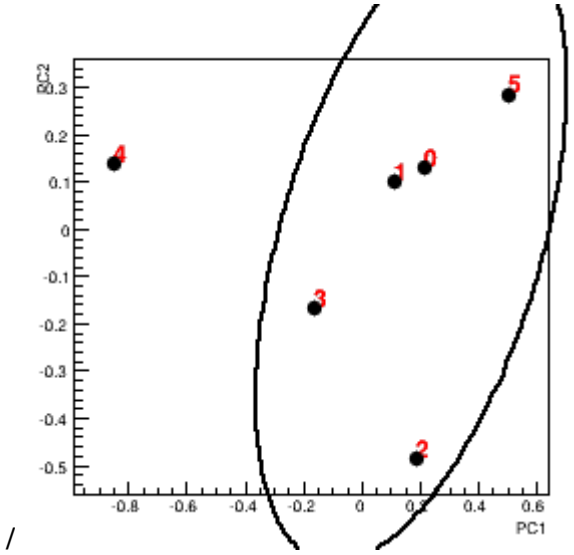


Fig.3 Scores

The score plot in Fig.3 shows that pure phase spectra (samples 4 and 5) are well separated by PC1, and intermediate samples taken at different times (0,1,2,3) are located in between the pure phase spectra. Sample 3, in particular, is intermediate between Cr(III) and Cr(VI), thus seems to consists in a 50% mixture of these pure phases.



/

Fig.4 Score plot PC1-PC2

The clustering algorithm applied to the PC1-PC2 space identifies two clusters: one constituted by the Cr(III) sample (n.4), the other by the Cr(VI) sample (n.5) and the mixtures Cr(VI)-Cr(III) formed at intermediate times during the reduction reaction (n.0-3). All the mixtures have shorter distance from Cr(VI) than from Cr(III), and their distance from n.5 is proportional to the measurement time.

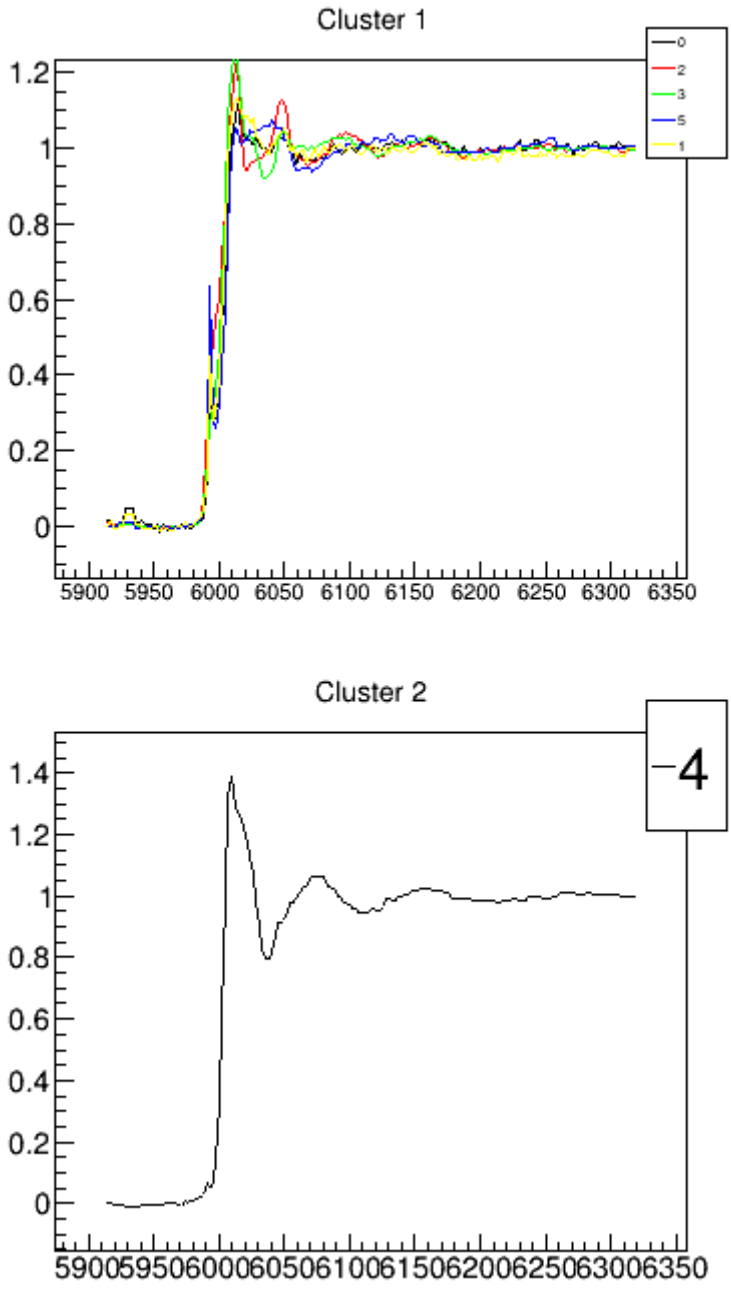


Fig.5 Spectra in clusters

The output file

The content of the output file named *outputXASFirstSight* is reported below, with comments added.

```
 Input from file: fileInputXASQualitative
------------------------------
  whichanalysis 1

  figpaper 1

  range 5000 6500
```

```
  threshold 0.96

  file  kinetic_01.extract

  file  kinetic_02.extract

  file  kinetic_03.extract

  file  kinetic04.extract

  file  cr3_2_exafs_s02_norm.nor

  purephase

  file  Cr6_exafs_2_flow_scan02_norm.nor

  purephase
```

The section above shows the commands read from the command file. It should be checked to ensure that they are interpreted correctly.

```
 Reading input files:
 ----------------------
Sample 0 -> file kinetic_01.extract
         Found 340  points
Sample 1 -> file kinetic_02.extract
         Found 340  points
Sample 2 -> file kinetic_03.extract
         Found 340  points
Sample 3 -> file kinetic04.extract
         Found 295  points
Sample 4 -> file cr3_2_exafs_s02_norm.nor
         Found 178  points
Sample 5 -> file Cr6_exafs_2_flow_scan02_norm.nor
         Found 170  points
```

The section above reports the number of data points read within each input file, as determined by the command *range*.

```
 Spectra have different binning

 Spectra interpolated to have equal binning

 Transforming input spectra to equal binning
Chosen range: [5914.76 6317.77]
Number of points: 171
```

The program realizes that spectra have different binning. A special procedure to interpolate the spectra to have equal binning is performed. In the section above the equalized range and points can be read.

```
 Starting Qualitative analysis

 n. points 171
Eigenvalues: 1 --> 70.60% (70.6%)
Eigenvalues: 2 --> 25.16% (95.8%)
Eigenvalues: 3 --> 2.43% (98.2%)
```

```
Eigenvalues: 4 --> 1.34% (99.5%)
Eigenvalues: 5 --> 0.49% (100.0%)
Eigenvalues: 6 --> 0.00% (100.0%)

 Chosen value of k=2: ratio=0.98 error=0.009
```

The section above shows the results of the PCA analysis. The first eigenvalues are listed as a function of their value, and the number of eigenvalues selected for PCA analysis is reported (k), together with the values of the threshold on the cumulative eigenvalue distribution (ratio), and an estimate of the corresponding error between original and reconstructed data (error). The threshold value is chosen on the basis of the command *threshold*.

```
 ============ Dendrogram =============
  Step      Dist     Sample 1    Sample 2
   5        1.07       0           4
   4        0.62       0           2
   3        0.47       2           3
   2        0.38       0           5
   1        0.11       0           1
 ====================================
 Normalized Cluster threshold: 0.200000 (0.763999)
 Normalized Cluster threshold redefined: (0.200000) 0.763999
 Cluster Threshold 0.844
```

The section above shows the dendrogram resulting from the hierarchical clustering. The value of the threshold distance chosen to define the number of clusters is reported.

```
 Cluster analysis

 Cluster 1 5)  0  2  3  5  1
 Cluster 2 1)  4
Cluster  1 PC0 center=0.17
Cluster  1 PC1 center=-0.03
Cluster  2 PC0 center=-0.85
Cluster  2 PC1 center=0.14

 Distances among clusters

 Cluster 1 Cluster 2 --> dist=1.04


 Cluster: 1
 Member: 1  Number: 0  File: kinetic_01.extract
 Member: 2  Number: 2  File: kinetic_03.extract
 Member: 3  Number: 3  File: kinetic04.extract
 Member: 4  Number: 5  File: Cr6_exafs_2_flow_scan02_norm.nor
 Member: 5  Number: 1  File: kinetic_02.extract

 Cluster: 2
 Member: 1  Number: 4  File: cr3_2_exafs_s02_norm.nor

 Cluster 1:  Representative spectrum: 1
 Cluster 2:  Representative spectrum: 4

 Cluster 1: Cluster population: 5  Representative spectrum: 1
 Cluster 2: Cluster population: 1  Representative spectrum: 4

 Cluster 1  Radius (0.75, 0.42)
```

The section above analyzes the formed clusters. The content of each cluster in terms of samples and file names, its center and Euclidean distance calculated in the PCA space, and the representative spectra of each cluster, corresponding to those nearest to its center, are listed. The cluster radius is calculated by using the Mahalanobis distance, and it is used to draw the 95% confidence ellipse.

Chapter 4

Quantitative analysis of XAS spectra

Motivation

Apply quantitative analysis by the MultiFit approach to spectra having variable binning and different binning and ranges among each other.

The command file

The list of commands is the following.

```
whichanalysis 3
varbin 1
figpaper 1
fitmodel 1
range 5000 6500
file  kinetic_01.extract
file  kinetic_02.extract
file  kinetic_03.extract
file  kinetic04.extract
file  cr3_2_exafs_s02_norm.nor
purephase
file  Cr6_exafs_2_flow_scan02_norm.nor
purephase
```

The commands have been included in the demo file named *fileInputXASQuantitative*. See the user guide for an explanation of their meaning.

Running RootProf

Start ROOT by clicking on his icon, or by typing “root” on a terminal window. Then write the root command:

```
Root> .x RootProf.C(“fileInputXASQuantitative”)
```

or

```
Root> .> outputXASQuantitative
.x RootProf.C(“fileInputXASQuantitative”)
.>
```

After some seconds, graphic windows will start appearing on your screen, while text output will appear on the terminal window, or redirected in the file named *outputXASQuantitative*. When the run ends, the root prompt will appear again on the ROOT terminal, and you will be able to edit each single graphic window and read the output file by your text editor.

The graphic output

Graphic windows in Fig.1-6 show the result of the MultiFit procedure applied to all XAS spectra given in input. Each of them is treated separately, by using its own binning and range. Best fit models (red line), constituted by linear combination of Cr(VI) and Cr(III) pure phases, are superimposed to measured spectra (black line).

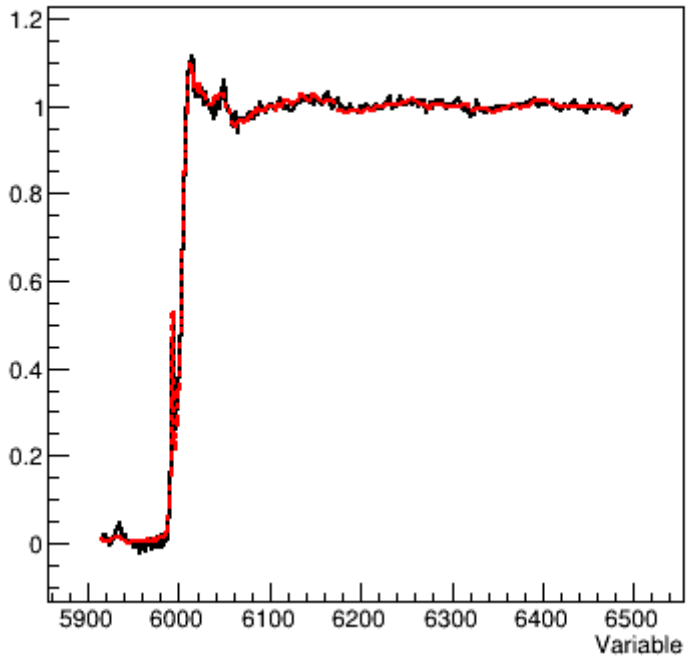


Fig.1 MultiFit on Sample 0

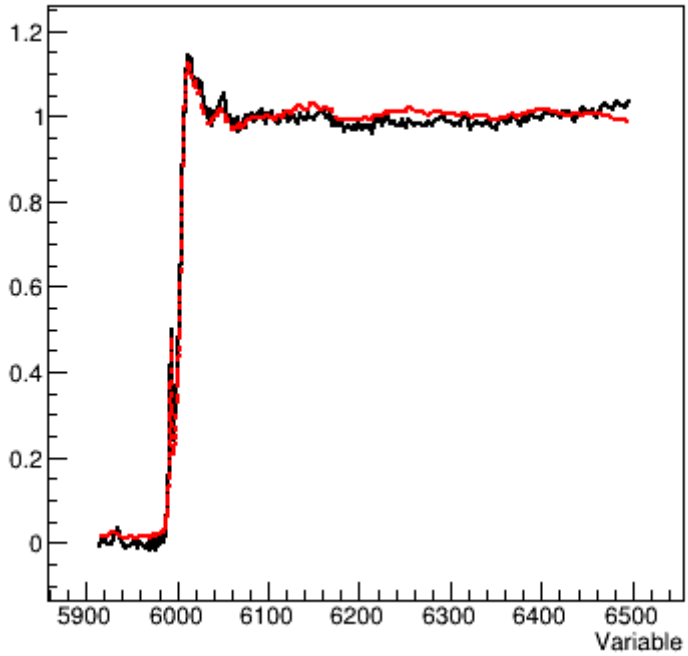


Fig.2 MultiFit on Sample 1

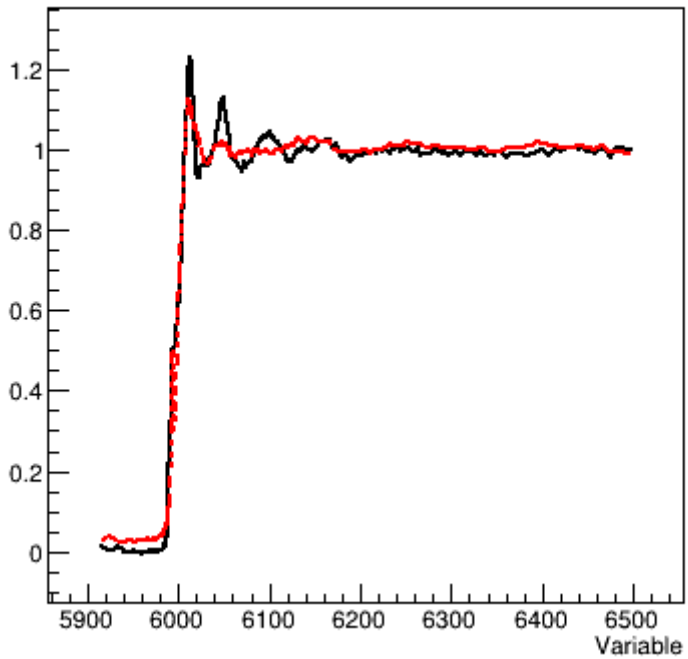


Fig.3 MultiFit on Sample 2

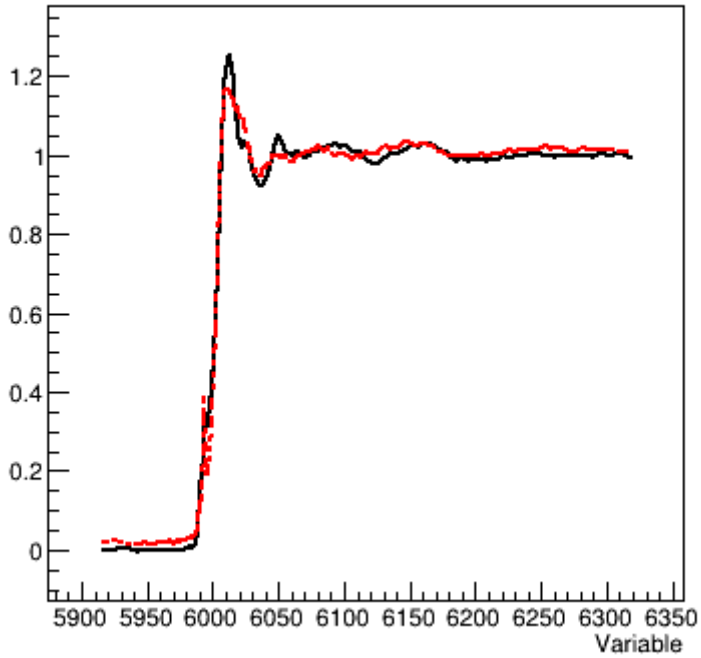


Fig.4 MultiFit on Sample 3

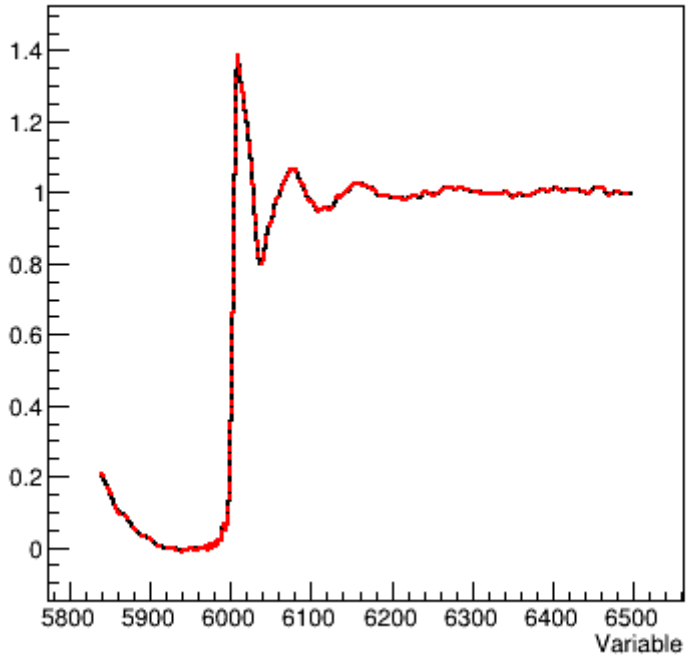


Fig.5 MultiFit on Sample 4

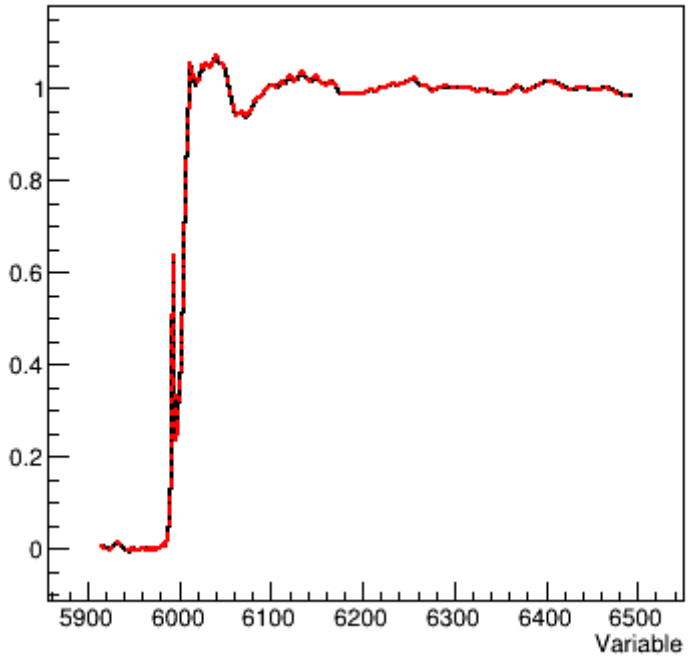


Fig.6 MultiFit on Sample 5

The fitting results are summarized in Fig.7, where the weight fractions of pure phases in the samples, as estimated by the fitting procedure, are plotted against the sample number. The sum of the two weight fractions (green line) deviates from unity, since no such constraint was used during fitting. It can be noted that the qualitative results obtained by PCA are confirmed: Samples 4 and 5 are pure phases, samples 0-3 have decreasing weight fraction of Cr(VI), sample 3 has nearly equal amount of Cr(III) and Cr(VI) phases.

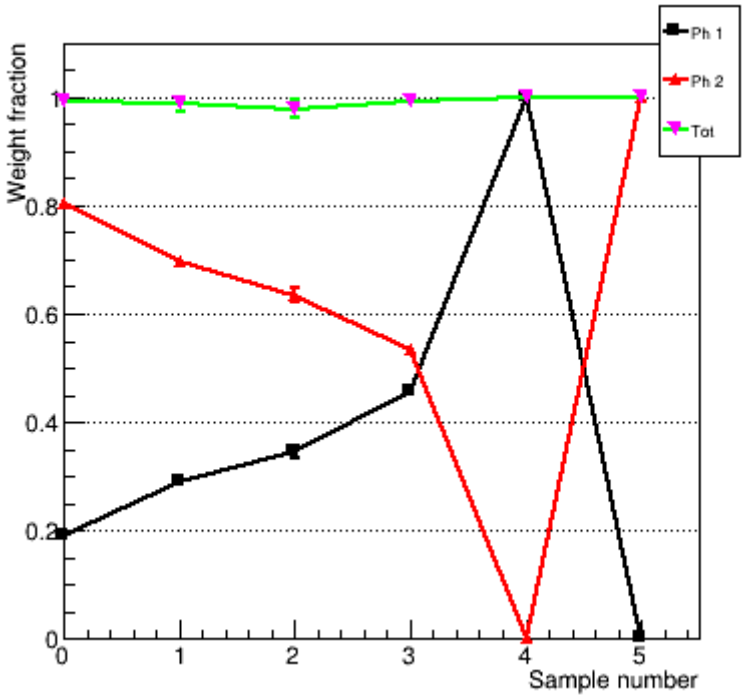


Fig.7 Quantitative Fit graph

The output file

The content of the output file named *outputXASQuantitative* is reported below, with comments added.

```
 Input from file: fileInputXASQuantitative
--------------------------------
  whichanalysis 3
  varbin 1
  figpaper 1
  fitmodel 1
  range 5000 6500
  file  kinetic_01.extract
  file  kinetic_02.extract
  file  kinetic_03.extract
  file  kinetic04.extract
  file  cr3_2_exafs_s02_norm.nor
  purephase
```

```
  file  Cr6_exafs_2_flow_scan02_norm.nor

  purephase
```

The above section shows the commands read from the command file. It should be checked to ensure that they are interpreted correctly.

```
Reading input files:
 ----------------------
Sample 0 -> file kinetic_01.extract
         Found 340  points
Sample 1 -> file kinetic_02.extract
         Found 340  points
Sample 2 -> file kinetic_03.extract
         Found 340  points
Sample 3 -> file kinetic04.extract
         Found 295  points
Sample 4 -> file cr3_2_exafs_s02_norm.nor
         Found 178  points
Sample 5 -> file Cr6_exafs_2_flow_scan02_norm.nor
         Found 170  points
```

The section above reports the number of data points read within each input file, as determined by the command *range*.

```
 Spectra have different binning
```

The program realizes that spectra have different binning. The special procedure to equalize binning and range of spectra is not performed, since the command *varbin* 1 is given in input..

```
Starting Quantitative analysis


 FIT RESULTS:
 ------------------------
 Spectrum 0: kinetic_01.extract
 Chi-Square=1.16e-01, Reduced Chi-Square=3.47e-04, NDF=334
 Weight fraction Phase 1  0.191 + 0.006
 Weight fraction Phase 2  0.802 + 0.006
 Total weight fraction 0.993 + 0.008
 ------------------------
 Spectrum 1: kinetic_02.extract
 Chi-Square=2.19e-01, Reduced Chi-Square=6.57e-04, NDF=334
 Weight fraction Phase 1  0.290 + 0.009
 Weight fraction Phase 2  0.697 + 0.010
 Total weight fraction 0.988 + 0.013
 ------------------------
 Spectrum 2: kinetic_03.extract
 Chi-Square=9.33e-01, Reduced Chi-Square=2.78e-03, NDF=335
 Weight fraction Phase 1  0.345 + 0.012
 Weight fraction Phase 2  0.634 + 0.013
 Total weight fraction 0.979 + 0.018
 FUNCTION MUST BE MINIMIZED BEFORE CALLING MINOs
 FUNCTION MUST BE MINIMIZED BEFORE CALLING MINOs
 ------------------------
```

```
Spectrum 3: kinetic04.extract
Chi-Square=4.33e-01, Reduced Chi-Square=1.49e-03, NDF=290
Weight fraction Phase 1  0.455 + 0.000
Weight fraction Phase 2  0.535 + 0.000
Total weight fraction 0.990 + 0.000
------------------------
Spectrum 4: cr3_2_exafs_s02_norm.nor
Chi-Square=5.05e-07, Reduced Chi-Square=2.92e-09, NDF=173
Weight fraction Phase 1  1.000 + 0.000
Weight fraction Phase 2  0.000 + 0.000
Total weight fraction 1.000 + 0.000
------------------------
Spectrum 5: Cr6_exafs_2_flow_scan02_norm.nor
Chi-Square=1.75e-05, Reduced Chi-Square=1.06e-07, NDF=165
Weight fraction Phase 1  0.003 + 0.002
Weight fraction Phase 2  0.997 + 0.000
Total weight fraction 1.000 + 0.002
```

The above section reports the results of the fitting procedure applied to each input spectrum separately. Fit results include goodness-of-fit estimates (Chi Square and Reduced Chi Square), number of degrees of freedom (NDF). The best fit estimates of the free parameters of the model (the weight fractions) is given, together with their sum (not contrained).

NOTE

If instead the *varbin* 1 command is commented in fileInputXASQuantitative, the MultiFit procedure is applied to equalized spectra. This speeds up the calculations, at the expense of the precision. In fact the fitting results obtained are shown in Fig.8.

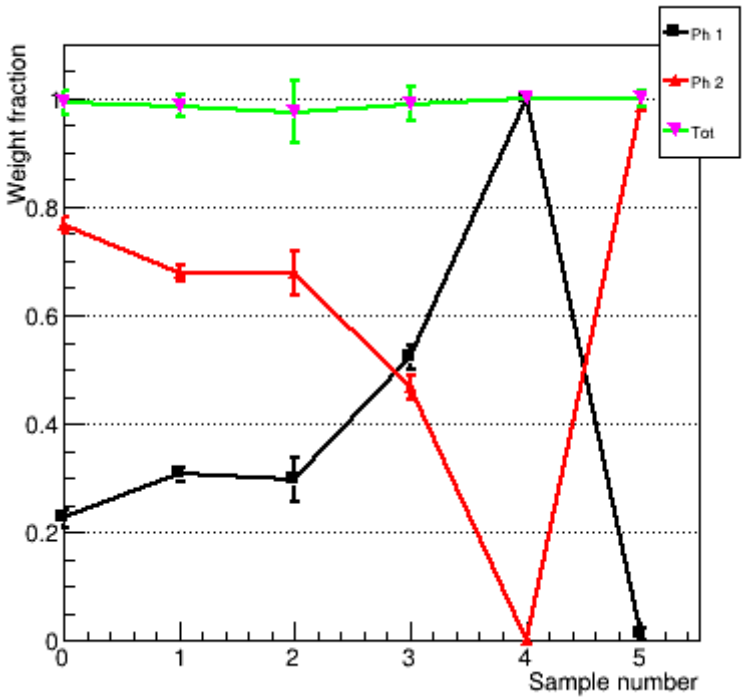


Fig.8 Quantitative Fit graph

Although the general trend of Fig.7 is confirmed, the weight fractions of samples 0-3 are no more proportional to the measurement time, with sample 1 and 2 having nearly the same estimated composition. This indicates that the interpolation procedure to equalize specta introduces uncertainties in quantitative analysis.