ChemoSpec: An R Package for Chemometric Analysis of Spectroscopic Data

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Abstract

ChemoSpec[1] is a collection of functions for plotting spectra (NMR, IR etc) and carrying out various forms of exploratory data analysis, such as hierarchical cluster analysis (HCA) and principal components analysis (PCA). Robust methods appropriate for this type of high-dimensional data are employed. ChemoSpec is designed to facilitate comparison of samples from treatment and control groups. It is designed to be user friendly and suitable for people with limited background in R. This vignette gives some background on ChemoSpec and takes the reader through a typical workflow.

1 Introduction

Chemometrics, as defined by Varmuza and Filzmoser[2], is

> "...the extraction of relevant information from chemical data by mathematical and statistical tools."

This is an appropriately broad definition, considering the wealth of questions and tasks that can be treated by chemometric approaches. In our case, the focus is on spectral data sets, which typically have many variables (frequencies) and relatively few samples. Such multivariate, emphhigh p, low n data sets present some algorithmic challenges, but these have been addressed by knowledgeable folks. In particular, for both the practical and theoretical background to multivariate chemometric analysis, I strongly recommend the Varmuza/Filzmoser book. A number of the functions described here are not much more than wrappers for the functions they have made available to the R community in their packages.

ChemoSpec was developed for the chemometric analysis of spectroscopic data, such as UV-Vis, NMR or IR data. I developed it while beginning a new research focus on plant metabolomics, and I needed software to analyze the data I was collecting. Part of my research involves ecological experiments on plant stress, so ChemoSpec was designed to accommodate samples that have different histories, i.e., they fall into different classes, categories or groups. Examples would be treatment and control groups, or simply different specimens (red flowers vs. blue flowers). Since my research is done with undergraduates, who are true novices with R, ChemoSpec is designed to be as user friendly as possible, with plenty of error checking, helpful warnings and a consistent interface. It also produces graphics that are consistent in style and annotation, and are suitable for use in publications and posters. Careful attention was given to writing the documentation for the functions, but this vignette serves as the best starting point for learning data analysis with ChemoSpec.

*The development of ChemoSpec has been generously supported by DePauw University in the form of sabbatical funding and a Fisher Fellowship. Thanks!

The centerpiece of ChemoSpec is the Spectra object. This is the place where your data is stored and made available to R. Once your data in stored this way and checked, all analyses are easily carried out. ChemoSpec currently ships with one main built-in data set called CuticleIR. You will see in just a moment how to access it and inspect it.

I assume you have at least a bare-bones knowledge of R as you begin to learn ChemoSpec, and have a good workflow set up. For detailed help on any function discussed here, type ?function_name at the console.

Finally, some conventions for this document: names of R "objects" such as packages, functions, function arguments, and data sets are in typewriter font. The commands you issue at the console and the output are in *slanted typewriter font*. Names of files stored on a computer are shown in blue to distinguish them from R objects which are kept in RAM.

Note: there are only a few key references in this version of the vignette — more will be coming eventually...

2 A Sample Exploration

This sample exploration is designed to illustrate a typical ChemoSpec workflow. You may wish to put your versions of these commands into a script file that you can source as you go along. This way you can easily make changes, and it will all be reproducible. To do this, open a blank R document, and type in your commands. Save it as something like My First ChemoSpec.R. Then you can either cut and paste portions of it to the console for execution, or you can source the entire thing:

```
> source("My First ChemoSpec.R")
```

Alternatively, at the GitHub site where you found ChemoSpec there is a file called VigExamples.R which you can download and modify to your heart's content.

2.1 Getting Data into ChemoSpec

Currently, there is only one means of moving raw data sets into ChemoSpec, and that is the function getManyCsv (it is relatively easy to write analogous functions for other formats). This function assumes that your raw data files are formatted as .csv files, and contain only the data itself, in two columns. The first column should be the frequency values, and this column must be the same for all files (as it will be if these are data sets from the same instrument and experimental parameters). The second column should contain the intensity values. There should not be a header row. If your data set contains treatment and control groups, or any analogous class/group information, this information should be encoded in the file names. getManyCsv argument gr.crit will be the basis for a grep process on the file names, and from there, each file, representing a sample, will be assigned to a group and be assigned a color as well. If your samples don't fall into groups, that's fine too, but you still have to give gr.crit something to go on—just give it one string that is common to all the file names. Obviously, this approach encourages one to name the files as they come off the instrument with forethought as to how they will be analyzed, which in turn depends upon your experimental design. Nothing wrong with having a plan! Remember that getManyCsv acts on all .csv files it finds in a directory, so don't have any extra .csv files hanging around. The output of getManyCsv is a Spectra object, which is R-speak for a file, readable by R, that contains not only your data, but other information about the experiment, as provided by you via the arguments to getManyCsv.

Here's a typical example (we have to talk hypothetically because I don't have your data). Let's say you had a folder containing 30 NMR files of flower essential oils. Imagine that 18 of these were from one hypothetical subspecies, and 12 from another. Further, let's pretend that the question under investigation has something to do with the taxonomy of these two supposed subspecies, in other words, an investigation into whether or not they should be considered subspecies at all. If the files were named like this:

sspA1.csv ... sspA18.csv and sspB1.csv ... sspB12.csv

Then the following command should process the files and create the desired Spectra object:

```
> getManyCsv(gr.crit = c("sspA", "sspB"), gr.cols = c("red3", "dodgerblue4"),
+ freq.unit = "ppm", int.unit = "peak intensity", descrip = "Subspecies Study",
+ out.file = "subspecies")
```

This causes getManyCsv to read the file names for the strings sspA and sspB and use these to assign the samples into groups. Samples in sspA*.csv files will be assigned the color red3 and sspB*.csv will be assigned dodgerblue4 (see the help file for some thinking-ahead about colors; ?getManyCsv at the console). After running this command, a new file called subspecies.RData will be in your directory. Next you should do a little renaming:

```
> load("subspecies.RData") # grabs the data, but it is called "spectra" at this point
> SubspeciesNMR <- spectra # copies the spectra to a new R object called SubspeciesNMR
> save(SubspeciesNMR, file = "SubspeciesNMR.RData")
```

You can now use the operating system to trash subspecies.RData as you won't need it any longer. In the future, you can double-click the SubspeciesNMR.RData file to load it automatically, or use

```
> load("SubspeciesNMR.RData")
```

to accomplish the same thing.

Built-in Data Sets

As mentioned above, ChemoSpec comes with one main built-in data set, CuticleIR. This data set is a series of IR spectra of the cuticle (leaf surface) of the plant *Portulaca oleracea*. The data was taken by gently pinning the leaf against an ATR sampling device. Also included is a data set gasNIR which is the gasoline data set that is included with R, converted to Spectra format. During the conversion, the octane values were transformed from numeric values to membership in L, M, or H classes by dividing the octane values into terciles. The gasNIR data set does not separate well (so far) by any statistical method I have employed, so I use it mainly for testing.

Color and Symbol Options

Coming soon...

2.2 Preliminary Inspection of Data

One of the first things you should do, and this is very important, is to make sure your data is in good shape. First, you can summarize the data set you created, and verify that the data ranges etc look like you expect them to:

```
> sumSpectra(SubspeciesNMR)
```

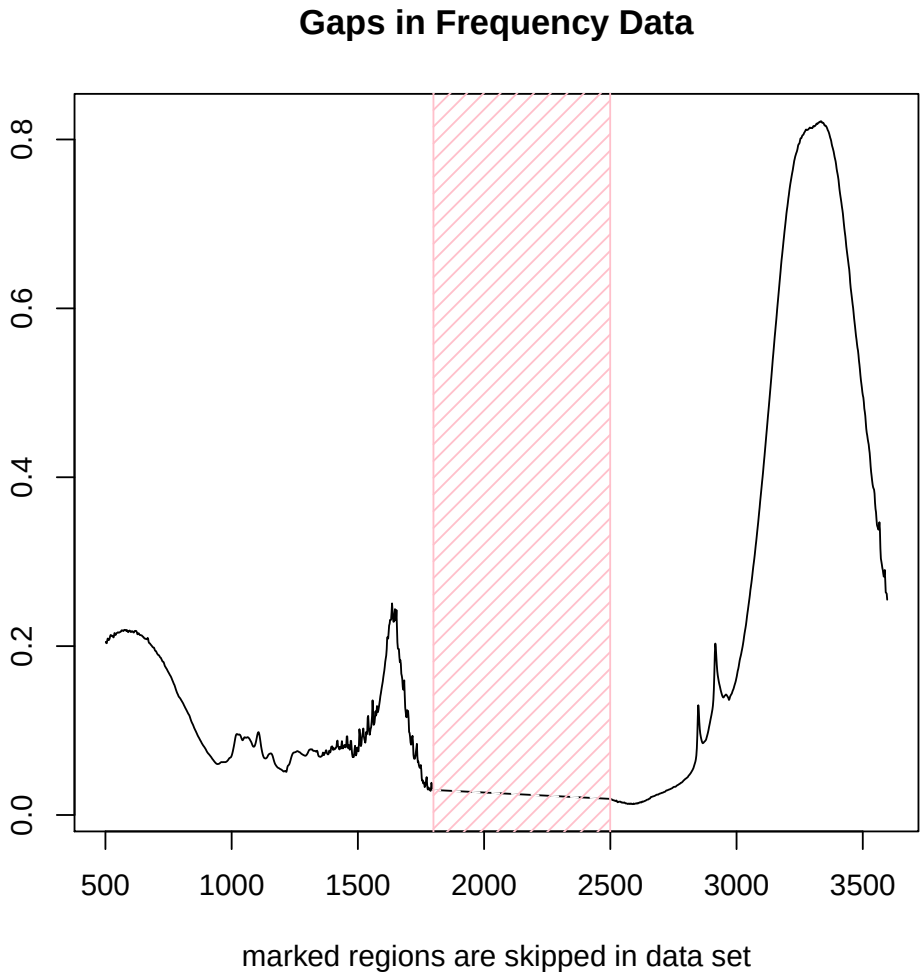
This will display quite a bit of information about your data set. Here are the commands and the result using the built-in data set CuticleIR:

```
> data(CuticleIR)
> sumSpectra(CuticleIR)
```

```
 Kelly's Complete IR Data Set, Summer 2009

        There are 157 spectra in this set.
        The y-axis unit is Absorbance.

        The frequency scale runs from 501.4261 to 3596.768 Wavenumbers
        There are 1242 frequency (x-axis) data points.
        The frequency resolution is 1.9286 Wavenumbers/point.

        This data set is not continuous along the frequency axis.
        Here are the data chunks:

   beg.freq end.freq     size beg.indx end.indx
1  501.4261 1797.420 1295.994        1      673
2 2501.3450 3596.768 1095.423      674     1242
```

Example 1: Procedure to Find Gaps in a Data Set

```
> check4Gaps(CuticleIR$freq, CuticleIR$data[1,], plot = TRUE)
   beg.freq end.freq     size beg.indx end.indx
1  501.4261 1797.420 1295.994        1      673
2 2501.3450 3596.768 1095.423      674     1242
```

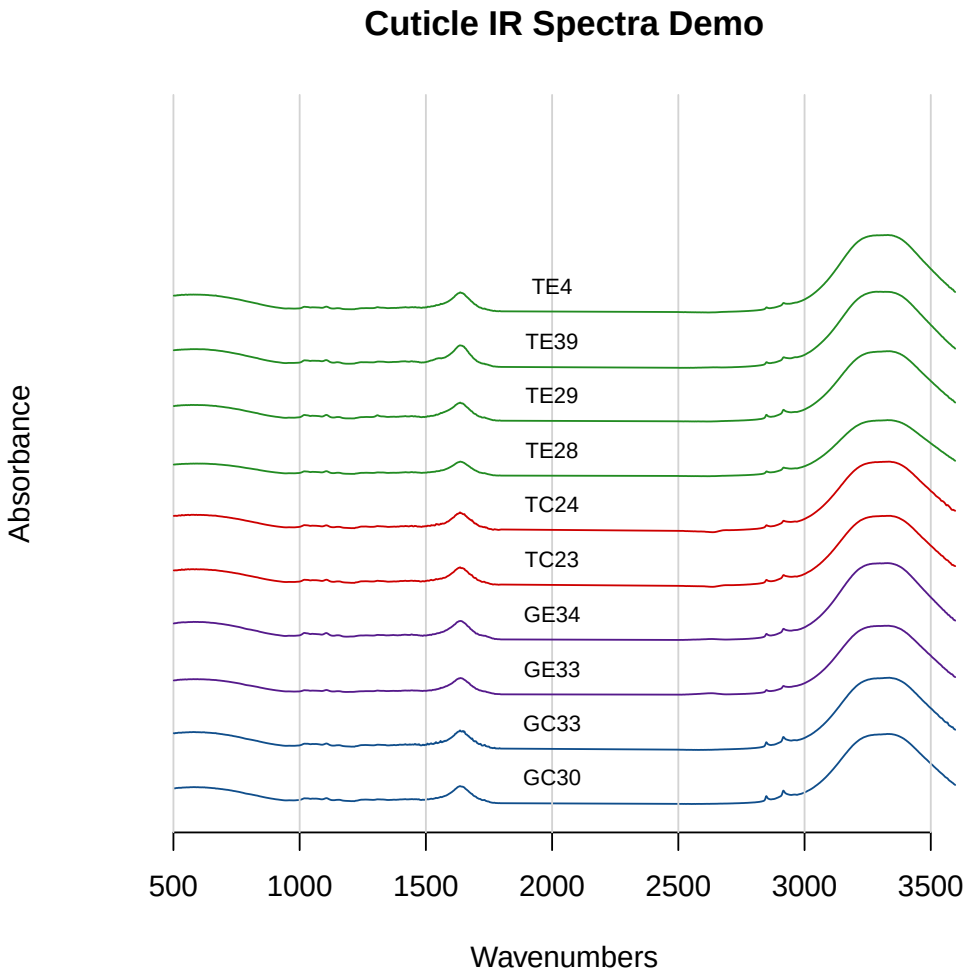



```
       The spectra are divided into 4 groups:

  group no.       color symbol alt.sym
1    GC  16 dodgerblue4      2       b
2    GE  35     purple4     15       d
3    TC  69        red3      1       a
4    TE  37 forestgreen      3       c

*** Note: this data is an S3 object of class 'Spectra'
```

Notice that `sumSpectra` has identified a gap in the data set; this is because a range of frequencies that contain no useful information were removed to compact the data—what's the point of analyzing baseline anyway? You can see this gap in the data as shown in Example 1 (`sumSpectra` checks for gaps, but doesn't produce the plot); both the numerical results and a figure are provided. Again, the point at this stage is to verify that your data looks like you expect it to. If there are problems, I'll tell you how to fix them in the next few sections.

Example 2: Plotting Spectra

```
> plotSpectra(CuticleIR, title = "Cuticle IR Spectra Demo",
+ which = c(10, 11, 40, 41, 100, 101, 140, 141, 150, 151),
+ yrange = c(0, 10), offset = 0.8, lab.pos = 2000)
```



2.2.1 Plotting the Spectra

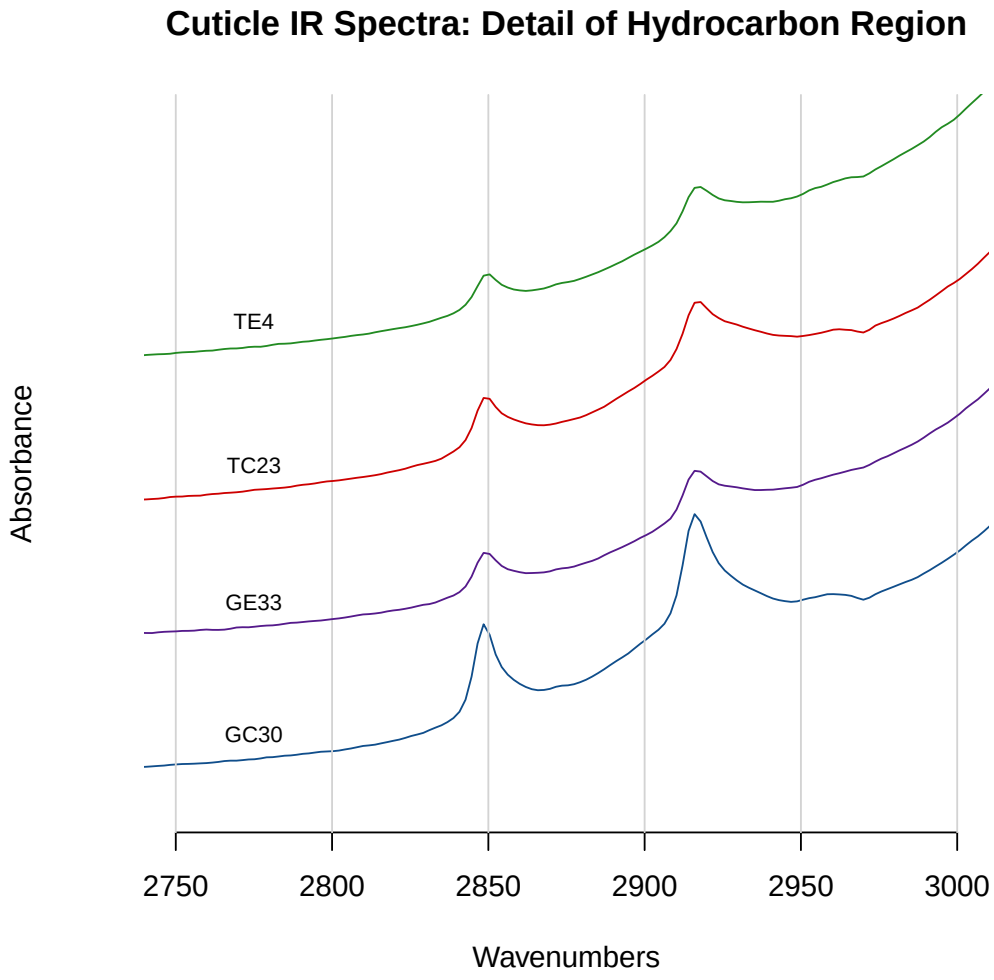
Assuming that everything looks good so far, it's time to plot the spectra and inspect them. Good practice would be to check every spectrum for artifacts and other potential problems, which might take a while; hopefully you looked at them when you originally recorded them. The basics of creating a plot are shown in Example 2.

Depending upon the intensity range of your data set, and the number of spectra to be plotted, you have to manually adjust the arguments `yrange`, `offset` and `amplify`, but this usually only takes a few iterations. Keep in mind that `offset`, and `amplify` are multiplied in the algorithm, so if you increase one, you may need to decrease the other. Suppose that you wanted to focus just on the hydrocarbon region of these spectra; you can add an argument called `xlim`. To demonstrate, let's look at fewer spectra, and at higher amplitude, so we can see details, as shown in Example 3.

The argument `which` in `plotSpectra` takes a numerical list of the spectra you wish to plot— you can think of this as the row number if you imagine each spectra to be a row in a matrix, with intensities in the columns (with each column corresponding to a particular frequency value). You may be wondering how to determine which particular sample is in each row. This is best accomplished with a grep command. For instance, if you wanted to know what row sample GE7 was in, the following command would locate it for you:

Example 3: Zooming in on a Spectral Region

```
> plotSpectra(CuticleIR, title = "Cuticle IR Spectra: Detail of Hydrocarbon Region",
+ which = c(10, 40, 100, 151), amplify = 10.0, xlim = c(2750, 3000),
+ yrange = c(0, 5), offset = 0.1, lab.pos = 2775)
```




```
> grep("GE7", CuticleIR$names)
[1] 49
```

See the discussion in the next section for more details on using grep effectively.

These sample plots display the IR spectra in two ways that may be upsetting to some readers: First, the x-axis is "backwards", because the underlying spectra were originally saved with an ascending frequency axis (which is not always the case). This is readily fixed by supplying the `xlim` argument in the desired order, e.g. `xlim = c(3000, 2750)` in the previous example. Second, the vertical scale in these examples is absorbance. When using IR for structural elucidation, the vertical axis is typically %T, with the peaks pointing downward. You don't have that choice in `ChemoSpec` because the absorbance mode is the appropriate one for chemometrics. Get used to it.

2.2.2 Identifying & Removing Problematic Samples

In the process of plotting and inspecting your spectra, you may find some spectra/samples that have problems. Perhaps they have instrumental artifacts. Or maybe you have decided to eliminate one subgroup of samples from your data

set to see how the results differ. To remove a particular sample, or samples meeting a certain criteria, you use the `removeSample` function. This function uses a grepping process based on its `rem.sam` argument, so you must be careful due to the greediness of grep. Let's imagine that sample TC138 has artifacts and needs to be removed. The command would be:

```
> noTC138 <- removeSample(CuticleIR, rem.sam = c("TC138"))
> sumSpectra(noTC138)

 Kelly's Complete IR Data Set, Summer 2009

        There are 156 spectra in this set.
        The y-axis unit is Absorbance.

        The frequency scale runs from 501.4261 to 3596.768 Wavenumbers
        There are 1242 frequency (x-axis) data points.
        The frequency resolution is 1.9286 Wavenumbers/point.

        This data set is not continuous along the frequency axis.
        Here are the data chunks:

   beg.freq end.freq     size beg.indx end.indx
1  501.4261 1797.420 1295.994        1      673
2 2501.3450 3596.768 1095.423      674     1242

        The spectra are divided into 4 groups:

  group no.       color symbol alt.sym
1    GC  16 dodgerblue4      2       b
2    GE  35     purple4     15       d
3    TC  68        red3      1       a
4    TE  37 forestgreen      3       c

*** Note: this data is an S3 object of class 'Spectra'
```

The `sumSpectra` command is optional, but confirms that there are now one fewer spectra in the set. You could also re-grep for the sample name to verify it is not found. If you wanted to remove sample TE2, and you tried to remove it this way, you would end up removing 10 spectra, as the string TE2 occurs in quite a few places. You can check this in advance with the grep function itself:

```
> TE2 <- grep("TE2", CuticleIR$names)
> CuticleIR$names[TE2] # gives the name(s) found

 [1] "TE2"  "TE20" "TE21" "TE22" "TE23" "TE24" "TE25" "TE26" "TE27" "TE28"
[11] "TE29"

> TE2 # gives the corresponding indicies

 [1] 131 132 133 134 135 136 137 138 139 140 141
```

This is what is meant by "grep is greedy". In this situation, you have three choices:

1. You could manually remove the problem samples (`> str(CuticleIR)` would give you an idea of how to do that; see also below under Hierarchical Cluster Analysis).
2. `removeSample` also accepts indices of samples, so you could grep as above, note the index of the sample you actually want to remove, and use that in `rem.sam`.
3. If you know a bit about grep, you can pass a more sophisticated search pattern to `rem.sam`. For instance, in the example above where we wanted to remove TE2, but "caught" some other sample names, you could change the search pattern as follows, which tells grep to find a string that ends in TE2 (so it could still begin with xyzTE2 and be found):

```
> newTE2 <- grep("TE2\\>",  CuticleIR$names)
> CuticleIR$names[newTE2] # gives the name(s) found
[1] "TE2"
> newTE2 # gives the corresponding indices
[1] 131
```

You can also take advantage of this greediness. For instance, using `rem.sam = c("TC")` would remove all the samples in this category, regardless of any other characters in the string, as long as T and C are next to each other (this goes back to planning your experiments, and how clever you were in naming your samples).

2.2.3 Identifying & Removing Regions of No Interest

Many spectra will have regions that should be removed. It may be an uninformative, interfering peak like the water peak in ^{1}H NMR, or the CO_2 peak in IR. Or, there may be regions of the spectra that simply don't have much information – they contribute a noisy baseline and not much else. An example would be the region from about 1,800 or 1,900 cm^{-1} to about 2,500 cm^{-1} in IR, a region where there are typically no peaks except for the CO_2 stretch, and rarely (be careful!) alkyne stretches.

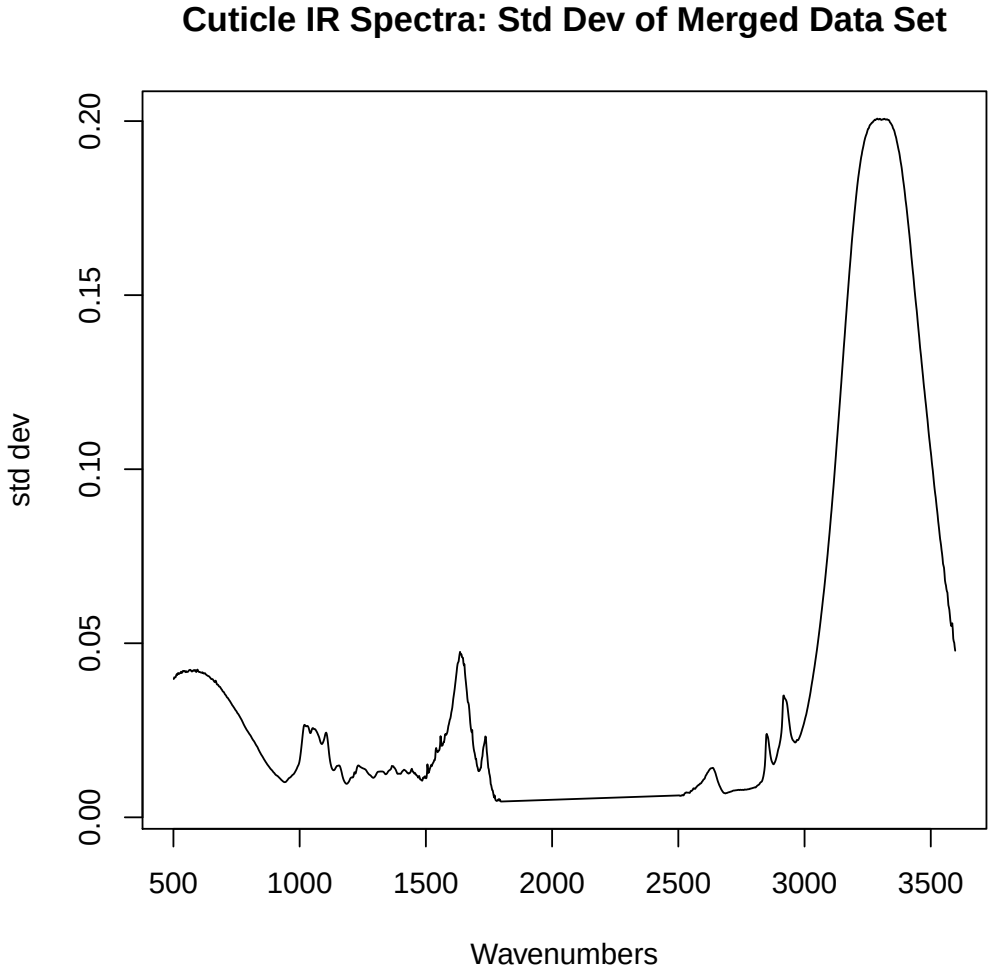
Finding these regions might be pretty simple, a matter of inspection coupled with your knowledge of spectroscopy. Another approach is to use the function `specSurvey` to examine the entire set of spectra. This function computes the standard deviation of the intensities at a particular frequency across the data set, and plots this against frequency. Regions where there is not much variation in the intensity will show up as unvarying baseline, and these regions are candidates for removal. Example 4 demonstrates the process.

Compare this to the figure produced by `check4Gaps` (Example 1); you'll see that the gap region is rendered by `specSurvey` by connecting the endpoints of each data chunk, producing what appears to be a region of little interest. All other areas have varying standard deviations, with the OH peak dominating, and no regions seem uninteresting, so let's keep them for now. However, if you wanted to remove certain peaks, the function `removeFreq` is the tool to use. In the `CuticleIR` data set, the water peak (above about 3,100 cm^{-1}) varies quite a bit, and may be just an artifact. We could remove it by doing the following. First, we need the upper end of the frequency range, which we can obtain with `sumSpectra` demonstrated above. That value is 3,597 cm^{-1} after rounding. Now, issue the following commands, ending with `sumSpectra` to verify what you wanted to happen, happened. Note that there are fewer frequency points now, and they end at approximately the value you specified (due to rounding).

```
> noOH <- removeFreq(CuticleIR, rem.freq = c(3100:3597))
> sumSpectra(noOH)

 Kelly's Complete IR Data Set, Summer 2009

        There are 157 spectra in this set.
        The y-axis unit is Absorbance.

        The frequency scale runs from 501.4261 to 3097.271 Wavenumbers
        There are 983 frequency (x-axis) data points.
        The frequency resolution is 1.9286 Wavenumbers/point.

        This data set is not continuous along the frequency axis.
        Here are the data chunks:

   beg.freq end.freq     size beg.indx end.indx
1  501.4261 1797.420 1295.994        1      673
2 2501.3450 3097.271  595.926      674      983

        The spectra are divided into 4 groups:

  group no.       color symbol alt.sym
1    GC  16 dodgerblue4      2       b
```

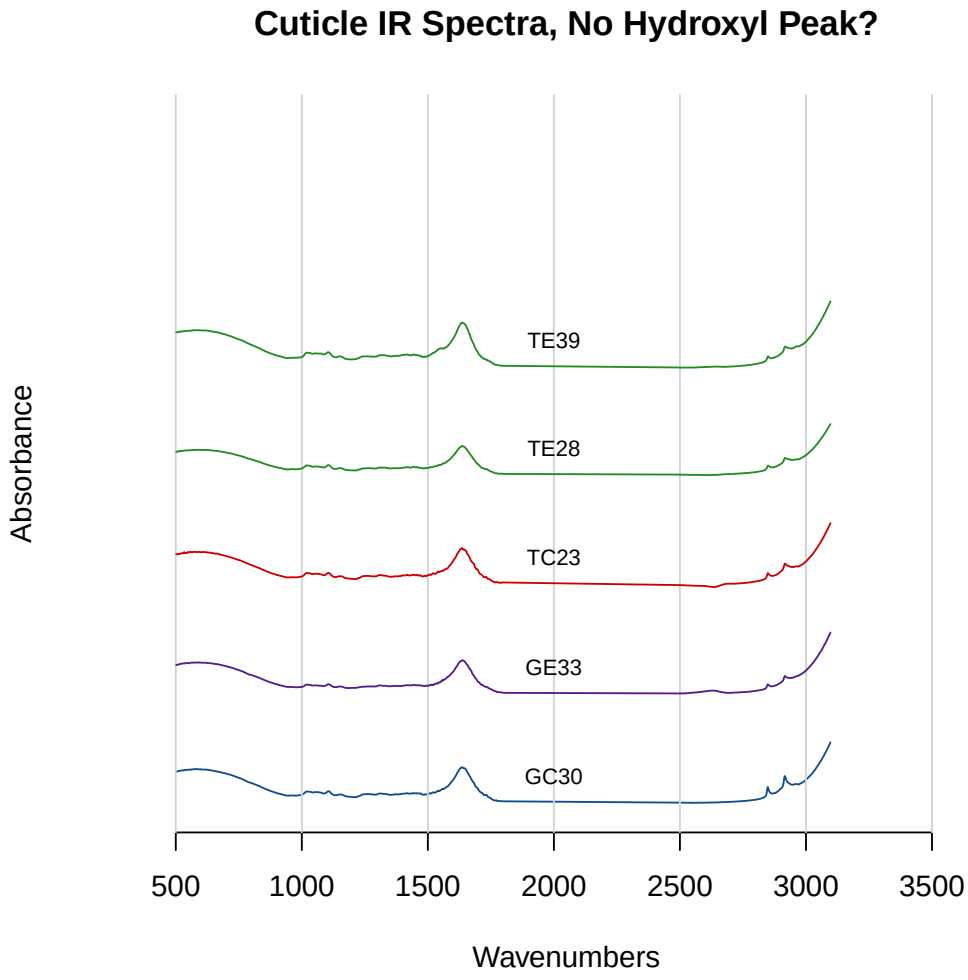
Example 4: Checking for Regions of No Interest

```
> specSurvey(CuticleIR, title = "Cuticle IR Spectra")
```



Example 5: Verifying removeFreq Worked as Desired

```
> plotSpectra(noOH, title = "Cuticle IR Spectra, No Hydroxyl Peak?",
+ which = c(10, 40,  100,  140 , 150),
+ yrange = c(0, 5), offset = 0.8, lab.pos = 2000, xlim = c(500, 3600))
```




```
2    GE  35     purple4     15       d
3    TC  69        red3      1       a
4    TE  37 forestgreen      3       c

*** Note: this data is an S3 object of class 'Spectra'
```

You could also plot the new spectra if desired, to make absolutely certain you removed what you wanted. In this case, we can make the point more strongly by plotting with `xlim` corresponding to the original data range, not that of the modified spectra, as shown in Example 5. We indeed did accomplish what we set out to do, though we might consider trimming off more frequencies, perhaps down to 3,000 cm^{-1}. You could also run `check4Gaps` as another check.

2.3 Data Pre-Processing Options

There are a number of data pre-processing options available for your consideration. The main choices are whether to normalize the data, whether to bin the data, and whether to scale the data. Data scaling is handled by the PCA routines, see Section 2.5. Normalization is handled by the `normSpectra` function. Usually one normalizes data in which the sample

preparation procedure may lead to differences in concentration, such as body fluids that might have been diluted during handling, or that vary due to the physiological state of the organism studied. The CuticleIR data set is taken using an ATR device in which the leaves are pinned against the IR analyzer, and no dilution is possible, so normalization isn't really appropriate. Currently, there is only one means of normalizing, and that is to divide each point in a spectrum by the sum of all points in that spectrum. Other means of normalizing can be readily added if they affect all points in the same way. Normalization is accomplished by:

```
> normCIR <- normSpectra(CuticleIR)
```

But remember, this is probably nonsensical for this data set.

Another type of pre-processing that you may wish to consider is binning or bucketing, in which groups of frequencies are collapsed into one frequency value, and the corresponding intensities are summed. There are two reasons for doing this. One is to compact the data, but the algorithms in R are quite fast, and data sets of the size of CuticleIR don't slow it down much. The other reason is to compensate for shifts in very narrow peaks from sample to sample. This is typically done in ^{1}H NMR because changes in dilution, ionic strength, or pH can cause slight shifts. Spectra with broad, rolling peaks won't have this problem (UV-Vis for example). The function binBuck is your friend:

```
> smallCIR <- binBuck(CuticleIR, bin.ratio = 4)

To preserve the requested bin.ratio,  1  data point(s)
has(have) been removed from the beginning of the data chunk 1

A total of 1 data points were removed to preserve the requested bin.ratio
To preserve the requested bin.ratio,  1  data point(s)
has(have) been removed from the beginning of the data chunk 2

A total of 2 data points were removed to preserve the requested bin.ratio

> sumSpectra(smallCIR)

 Kelly's Complete IR Data Set, Summer 2009

        There are 157 spectra in this set.
        The y-axis unit is Absorbance.

        The frequency scale runs from 506.2475 to 3593.875 Wavenumbers
        There are 310 frequency (x-axis) data points.
        The frequency resolution is 7.71425 Wavenumbers/point.

        This data set is not continuous along the frequency axis.
        Here are the data chunks:

   beg.freq end.freq     size beg.indx end.indx
1  506.2475 1794.527 1288.279        1      168
2 2506.1663 3593.875 1087.709      169      310

        The spectra are divided into 4 groups:

  group no.       color symbol alt.sym
1    GC  16 dodgerblue4      2       b
2    GE  35     purple4     15       d
3    TC  69        red3      1       a
4    TE  37 forestgreen      3       c

*** Note: this data is an S3 object of class 'Spectra'
```

Compare the results here with the sumSpectra of the full data set (Section 2.2. In particular note that the frequency resolution has gone down due to the binning process.

2.4 Hierarchical Cluster Analysis

Hierarchical cluster analysis (HCA from now on) is a clustering method (surprise!) in which "distances" between samples are calculated and displayed in a dendrogram (a tree-like structure; these are also used in evolution and systematics where they are called cladograms). The details behind HCA can be readily found elsewhere (Chapter 6 of [2] is a good choice). With `ChemoSpec` you have access to any of the methods available in `R` function `hclust`; for more details, type `?hclust` at the console. To demonstrate, let's first remove some samples from `CuticleIR` so the resulting plot is a bit less cluttered. Then we'll carry out the HCA itself. The sample removal will be done manually; this process and the results are shown in Example 6.

The result is a dendrogram. The vertical scale represents the numerical distance between samples. For this data set, there is no obvious clustering by group. Note that the function `hcaScores` does the same kind of analysis using the results of PCA, rather than the raw spectra. It is discussed in the next section.

2.5 Principal Components Analysis

Principal components analysis (PCA from now on) is the real workhorse of exploratory data analysis. It makes no assumptions about group membership, but clustering (possibly in high dimensions) of the resulting sample scores can be very helpful in understanding your data. The theory and practice of PCA is covered well elsewhere (Chapter 3 of [2] is an excellent choice). Here, we'll concentrate on using the PCA methods in `ChemoSpec`. Briefly however, you can think of PCA as determining the minimum number of components necessary to describe a data set, in effect, removing noise. Think of a typical spectrum: some regions are clearly just noise. Further, a typical spectroscopic peak spans quite a few frequency units as the peak goes up, tops out, and then returns to baseline. Any one of the points in a particular peak describe much the same thing, namely the intensity of the peak. Plus, each frequency within a given peak envelope is correlated to every other frequency in the envelope (they rise and fall in unison as the peak changes size from sample to sample). PCA can look "past" all the noise and correlation in the data set, and boil the entire data set down to essentials. Unfortunately, the principal components that are uncovered in the process don't correspond to anything concrete, usually. Again, you may wish to consult a more detailed treatment!

Table 1 gives an overview of the options available in `ChemoSpec`, and the relevant functions.

There's quite a bit of choice here; let's work through an example and illustrate, or at least mention, the options as we go. Keep in mind that it's up to you to decide how to analyze your data. Most people try various options, and follow the ones that lead to the most insight. But the decision is yours!

The first step is to carry out the PCA. You have two main options, either classical methods, or robust methods. Classical methods use all the data you provide to compute the scores and loadings. Robust methods focus on the core or heart of the data, which means that some samples may be downweighted. This difference is important, and the results from the two methods may be quite different, depending upon your the nature of your data. The differences arise because PCA methods (both classical and robust) attempt to find the components that explain as much of the variance in the data set as possible. If you have a sample that is genuinely corrupted, for instance due to sample handling, its spectral profile may be very different from all other samples, and it can legitimately be called an outlier. In classical PCA, this one sample will contribute strongly to the variance of the entire data set, and the PCA scores will reflect that (it is sometimes said that scores and loadings follow the outliers). With robust PCA, samples with rather different characteristics do not have as great an influence, because robust measures of variance, such as the median absolute deviation, are used.

Besides choosing to use classical or robust methods, you also need to choose a scaling method. For classical PCA, your choices are no scaling, autoscaling, or Pareto scaling. In classical analysis, if you don't scale the data, large peaks contribute more strongly to the results. If you autoscale, then each peak contributes equally to the results (including noise "peaks"). Pareto scaling is a compromise between these two. For robust PCA, you can choose not to scale, or you can scale according to the median absolute deviation. Median absolute deviation is a means of downweighting larger peaks.

There is not enough space here to illustrate all possible combinations of options; Example 7 and Example 8 show the use and results of classical and robust PCA without scaling, followed by plotting of the first two PCs (we'll discuss plotting options momentarily). You can see from these plots that the robust and classical methods have produced rather different results, not only in the overall appearance of the plots, but in the amount of variance explained by each PC.

Example 6: Manual Removal of Samples Followed by HCA

```
> keep <- seq(1, 157, 6) # select every 6th spectrum
> someCIR <- CuticleIR # make a copy of the original data
> someCIR$names <- someCIR$names[keep] # modify each piece manually
> someCIR$colors <- someCIR$colors[keep]
> someCIR$groups <- someCIR$groups[keep]
> someCIR$sym <- someCIR$sym[keep]
> someCIR$alt.sym <- someCIR$alt.sym[keep]
> someCIR$data <- someCIR$data[keep,] # samples in rows, freq in columns
> chkSpectra(someCIR, confirm = TRUE) # make sure we didn't screw it up!
You must be awesome: These spectra look just dandy!
> hcaSpectra(someCIR, title = "Every 6th Sample of CuticleIR")
```

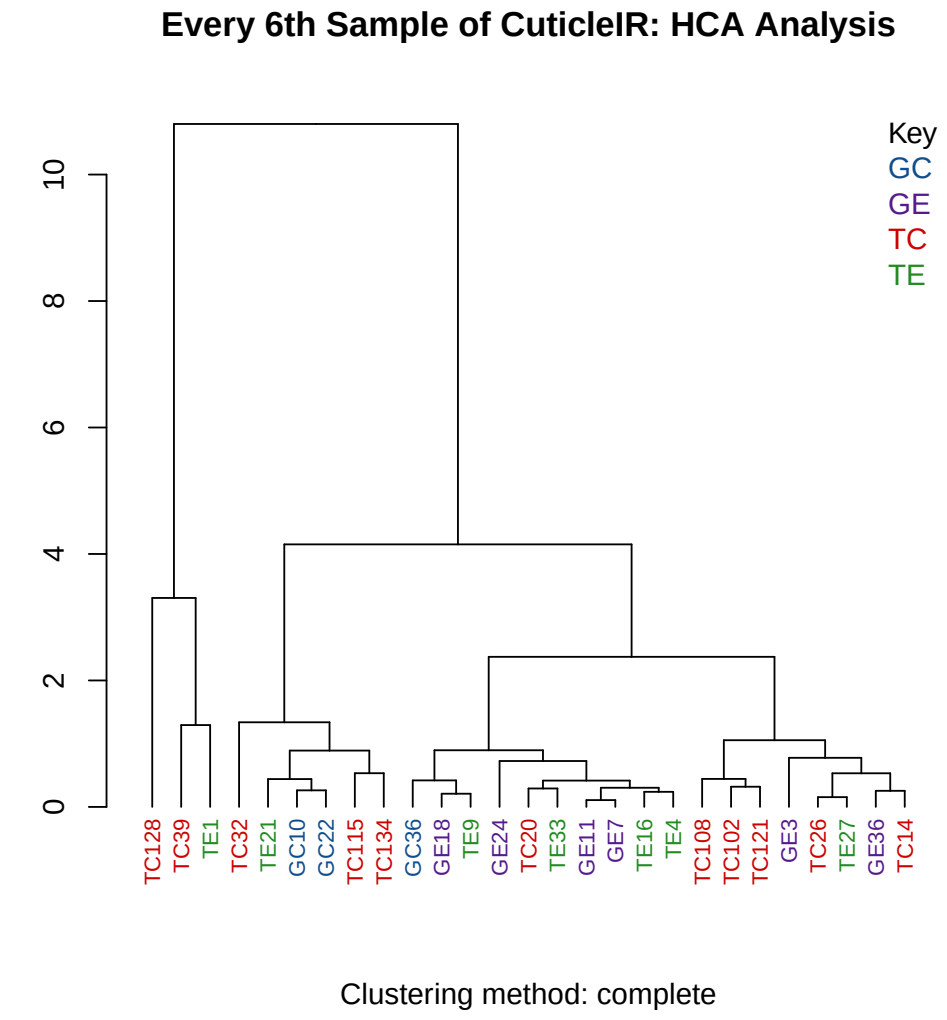


Table 1: Principal Components Analysis Options & Functions

PCA options	scaling options	function
classical PCA	no scaling, autoscaling, Pareto scaling	`classPCA`
robust PCA	no scaling, median absolute deviation	`robPCA`
Diagnostics		
OD plots		`pcaDiag`
SD plots		`pcaDiag`
Choosing the correct no. of PCs		
scree plot		`plotScree`
bootstrap analysis (classical PCA only)		`pcaBoot`
Score plots	plotting options	
2D plots	robust or classical confidence ellipses	`plotScores`
3D plots		
—static 3D plots		`plotScores3D`
—interactive 3D plots		`plotScoresRGL`
—interactive multivariate plots		`plotScoresG`
Loading plots		
loadings vs frequencies		`plotLoadings`
loadings vs other loadings		`plot2Loadings`
Other		
HCA of PCA scores		`hcaScores`

Since we've plotted the scores to see the results, let's mention a few features of `plotScores` which produces a 2D plot of the results (we'll deal with 3D options later). Note that an annotation is provided in the upper left corner of the plot that describes the history of this analysis, so you don't lose track of what you are viewing. The `tol` argument controls what fraction of points are labeled with the sample name. This is a means of identifying potential outliers. The `ellipse` argument determines if and how the ellipses are drawn (the 95% confidence interval is used).

You can choose `"none"` for no ellipses, `"cls"` for classically computed confidence ellipses, `"rob"` for robustly computed ellipses, or `"both"` if you want to directly compare the two. Note that the use of classical and robust here has nothing to do with the PCA algorithm — it's the same idea, but applied to the 2D array of scores. With the robust ellipses, points outside the ellipses are more likely candidates for outlier status.

Plots such as Figures 7 and 8 can give you an idea of potential outliers, but `ChemoSpec` includes more sophisticated approaches. The function `pcaDiag` can produce two types of plots that can be helpful (Figures 9 and 10). The meaning and interpretation of these plots is discussed in more detail in Varmuza and Filzmoser, Chapter 3[2].

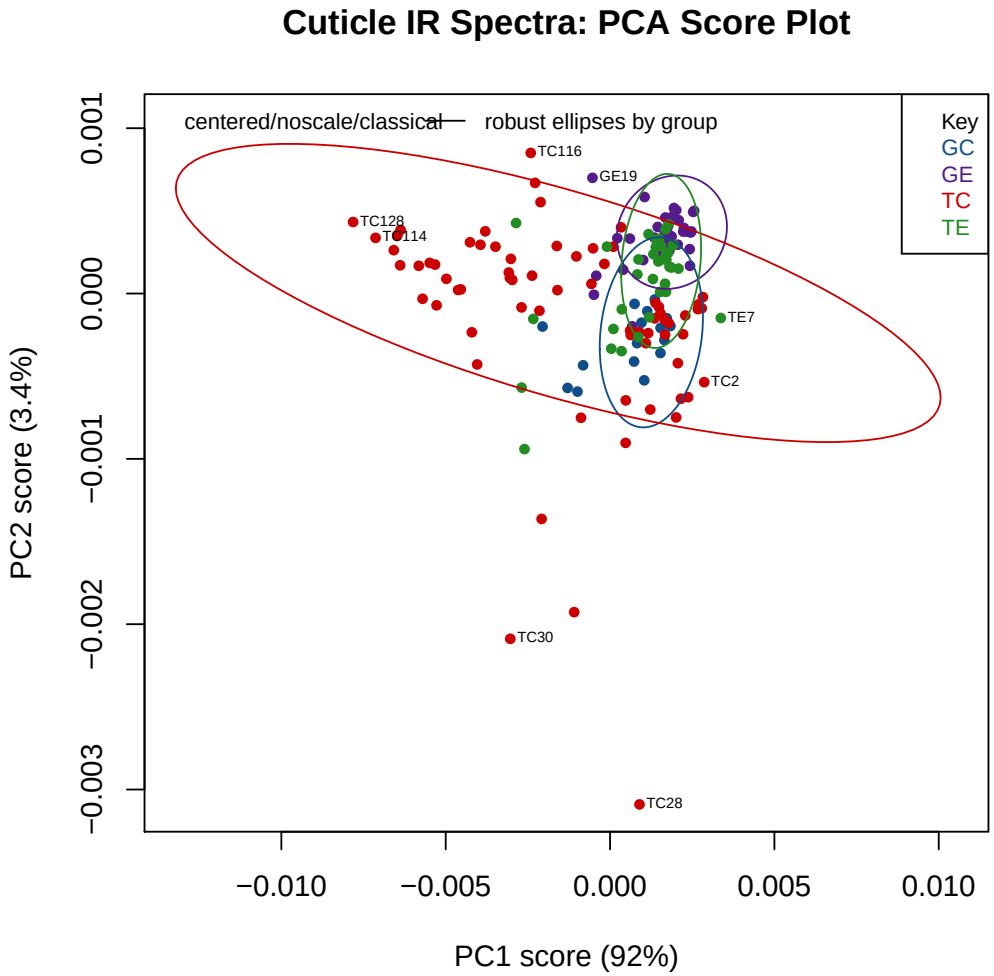
Depending upon your data, and your interpretation of the results, you may decide that some samples should be discarded, in which case you can use `removeSample` as previously described, then repeat the PCA analysis. The next step for most people is to determine the number of PCs needed to describe the data. This is usually done with a scree plot as shown in Example 11. In this case, it's clear that most of the variance can be described by one PC! We'll pursue that more in a bit.

If you are using classical PCA, you can also get a sense of the number of PCs needed via a bootstrap method, as shown in Example 12. Note that this method is iterative and takes a bit of time. Comparing these results to the scree plot, you'll see that the bootstrap method suggests that 2 PCs would not always be enough to reach the 95% level, while the scree plot suggests that 2 PCs is sufficient.

Now let's turn to viewing scores in 3D. There are currently 3 options in `ChemoSpec`: plotting using `lattice` graphics, which produces a static plot that you have to adjust manually, and two interactive plots, one based on package `rgl` and one based upon package `rggobi` which in turn uses the program `ggobi`. Probably the best place to start is with `plotScoresRGL`. It it well suited to exploring your data, and can be printed out in high quality. However, the nature of

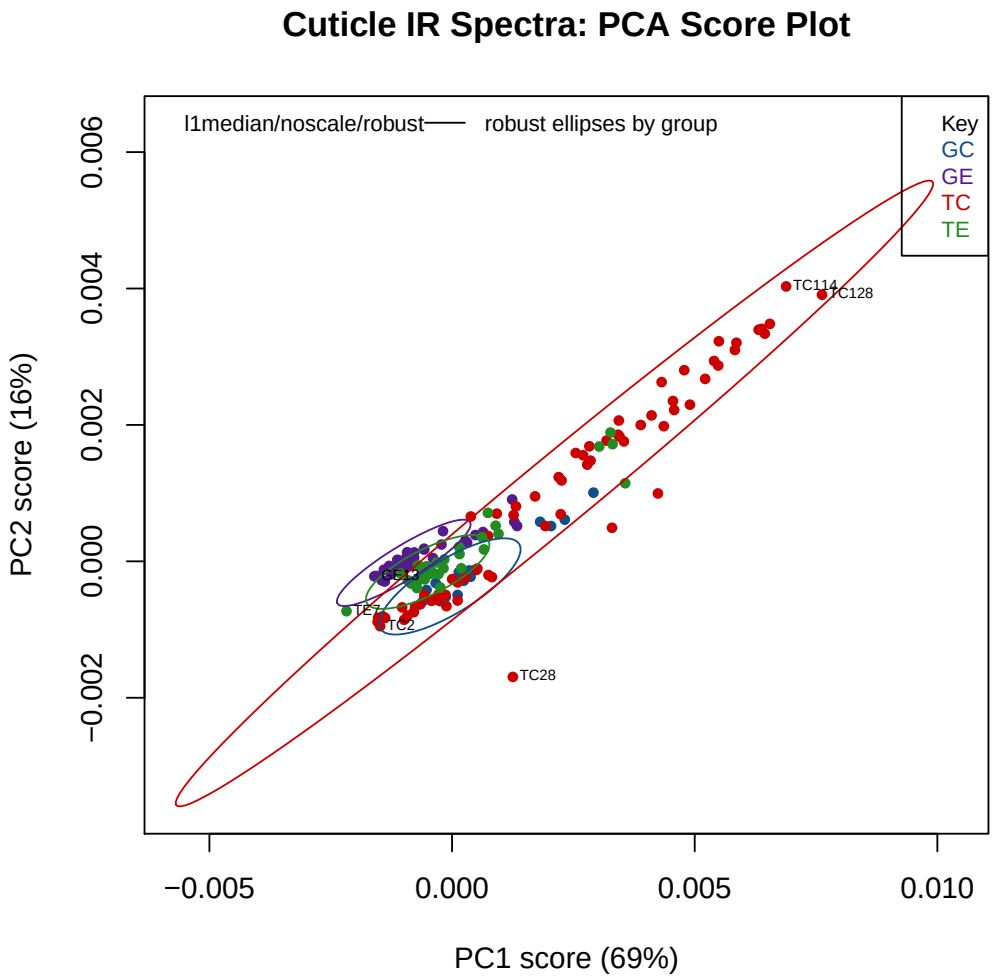
Example 7: How to Carry Out Classical PCA

```
> class <- classPCA(CuticleIR, choice = "noscale")
> plotScores(CuticleIR, title = "Cuticle IR Spectra", class,
+ pcs = c(1,2), ellipse = "rob", tol = 0.01)
```



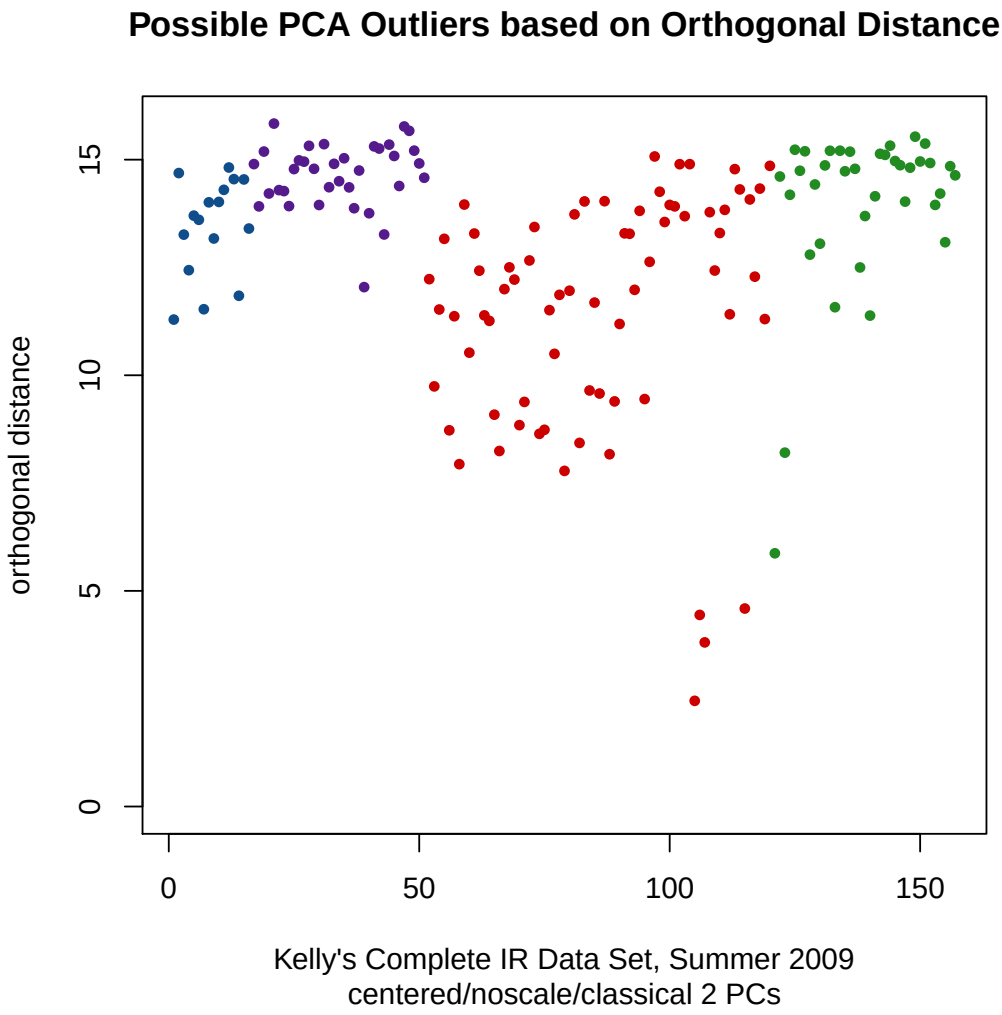
Example 8: How to Carry Out Robust PCA

```
> robust <- robPCA(CuticleIR, choice = "noscale")
> plotScores(CuticleIR, title = "Cuticle IR Spectra", robust,
+ pcs = c(1,2), ellipse = "rob", tol = 0.01)
```



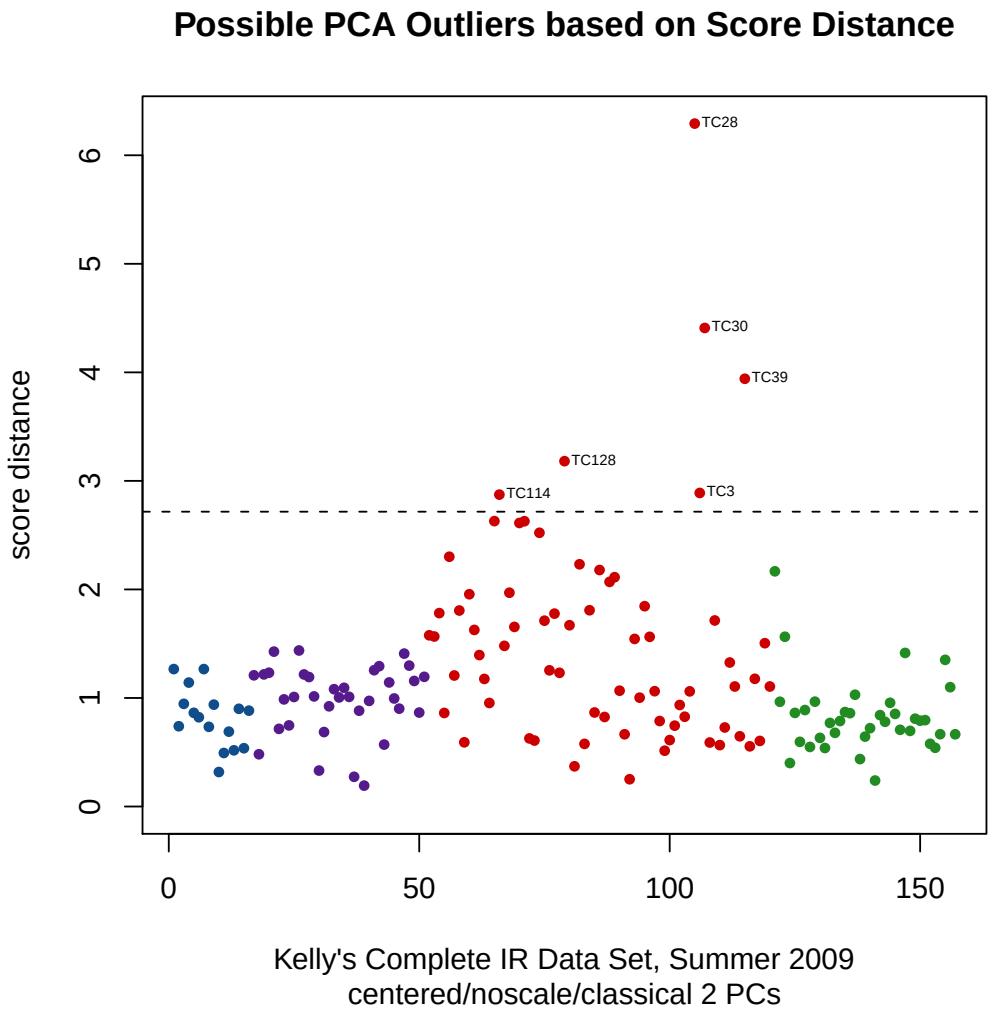
Example 9: Diagnostics: Orthogonal Distances

```
> diagnostics <- pcaDiag(CuticleIR, class, pcs = 2, plot = "OD")
```



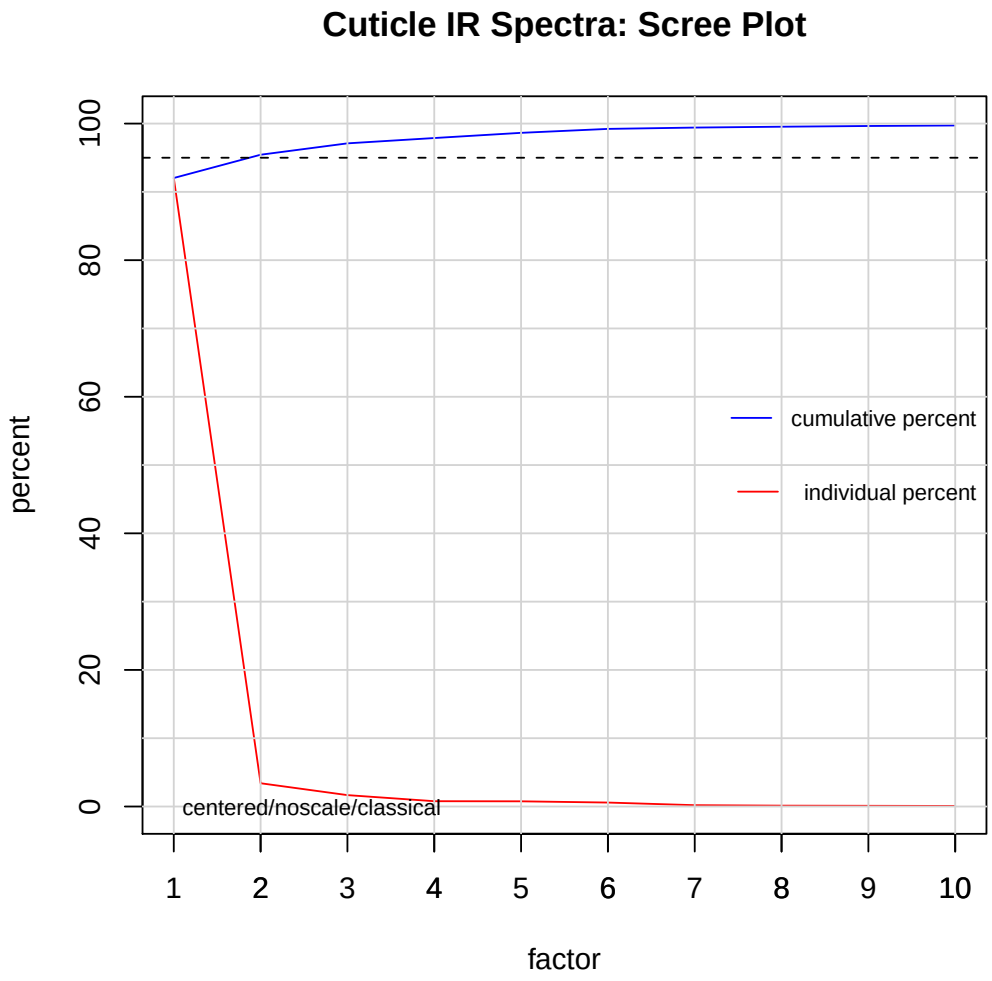
Example 10: Diagnostics: Score Distances

```
> diagnostics <- pcaDiag(CuticleIR, class, pcs = 2, plot = "SD")
```



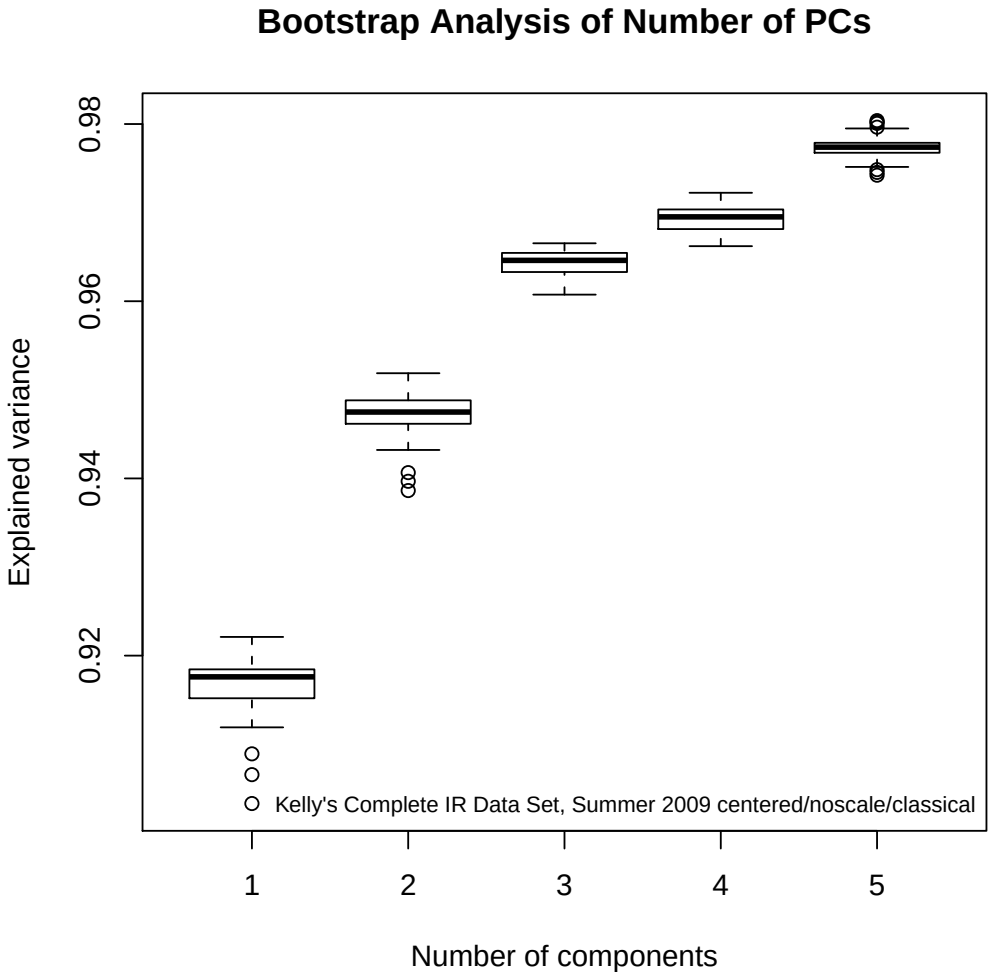
Example 11: Creating a Scree Plot

```
> plotScree(class, title = "Cuticle IR Spectra")
```



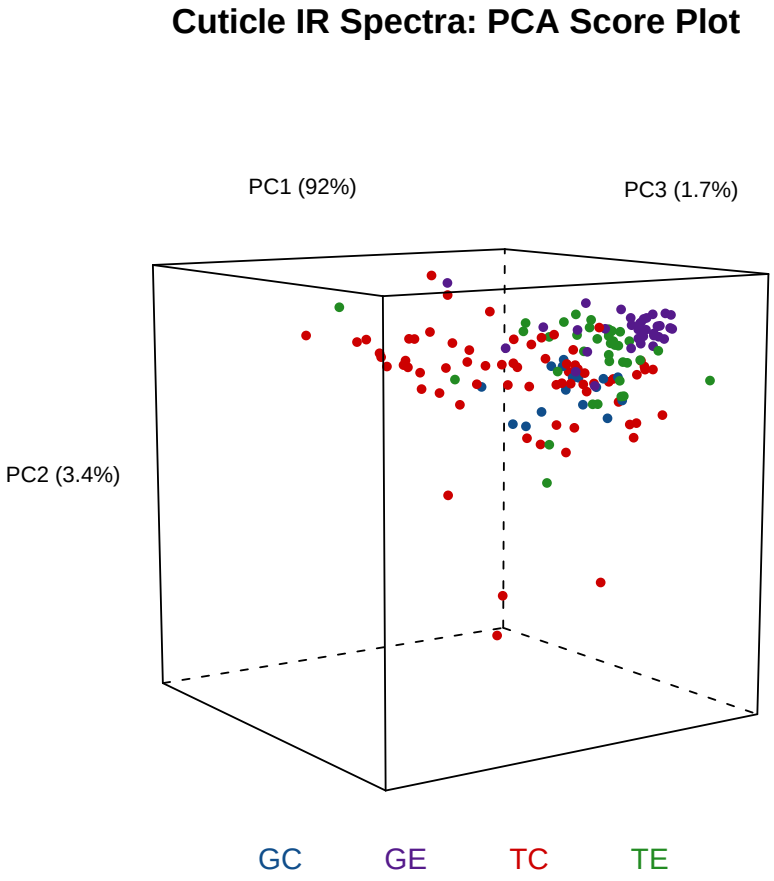
Example 12: Conducting Bootstrap Analysis for No. of PCs

```
> out <- pcaBoot(CuticleIR, pcs = 5, choice = "noscale")
```



Example 13: One Way to Plot Scores in 3D

```
> plotScores3D(CuticleIR, class, title = "Cuticle IR Spectra")
```



the GL graphics device means that the title and the legend move with the data, so this may not give a hardcopy suitable for publications. This interactive plot cannot be invoked in this document, but here are the necessary commands:

```
> plotScoresRGL(CuticleIR, class, title = "Cuticle IR Spectra",
+ leg.pos = "A", t.pos = "B") # not run - it's interactive!
```

For full details, of course take a look at the manual page, `?plotScoresRGL`. If you want a similar and probably more publication-worthy plot, you can use `plotScores3D` as shown in Example 13.

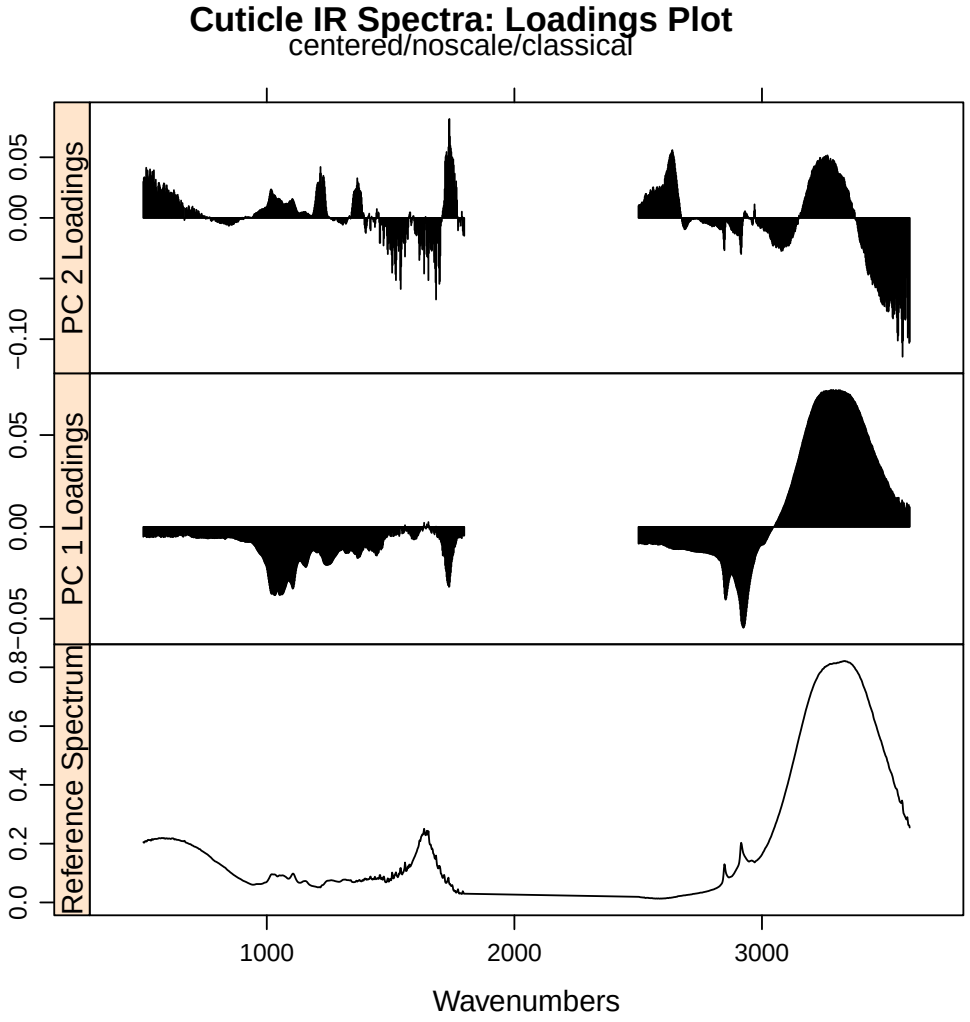
Finally, you can install the program `ggobi` and the package `rggobi` and use `plotScoresG` to produce an different interactive plot. This is actually the most powerful analysis tool, as `ggobi` is not restricted to 3 dimensions, and can use projection pursuit methods to find interesting views of your data. With an additional package, `DescribeDisplay`, you can create very nice plots of your data. Note that at this writing (December 8, 2009), `rggobi` is apparently waiting for an update, as it has disappeared from the CRAN servers.

```
> plotScoresG(CuticleIR, class) # not run - it's interactive!
```

In addition to the scores, PCA also produces loadings which tell you how each variable (frequencies in spectral applications) affect the scores. Examining these loadings can be critical to interpreting your results. Example 14 gives an example. You can see that the hydroxyl peak above 3,000 cm^{-1} has a large effect on PC 1, and it is this peak that is responsible for PC 1 explaining over 90% of the variance (mentioned earlier). It would be of interest to eliminate this peak, and repeat

Example 14: Creating a Loading Plot

```
> plotLoadings(CuticleIR, class, title = "Cuticle IR Spectra",
+ loads = c(1, 2), ref = 1)
```



the PCA, but analysis of specific data is not our goal here.

You can also plot one loading against another, using function `plot2Loadings` (Example 15). This is typically not too useful for spectroscopic data, since many of the variables are correlated (as they are parts of the same peak, hence the serpentine lines in the figure). The most extreme points on the plot, however, can give you an idea of which peaks (frequencies) serve to differentiate a pair of PCs, and hence, drive your data clustering.

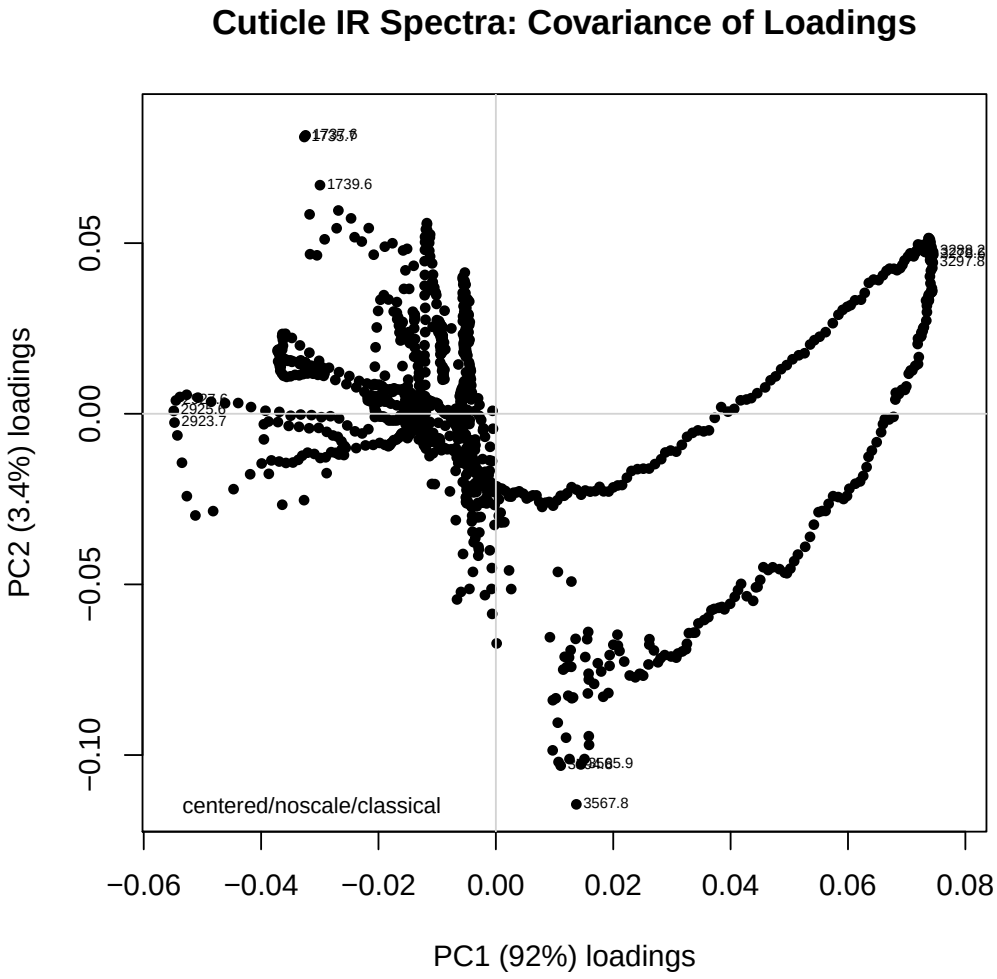
Finally, you can blend the ideas of PCA and HCA. Since PCA eliminates the noise in a data set (after you have selected the important PCs), you can carry out HCA on the PCA scores, since the scores represent the cleaned up data. The result using the `CuticleIR` data set are not much better than doing HCA on the raw spectra, so we won't illustrate it, but the command would be:

```
> hcaScores(CuticleIR,  class, scores = c(1:5), title = "Cuticle IR Spectra",
+ method = "complete")
```

I hope you have enjoyed this tour of the features of `ChemoSpec`!

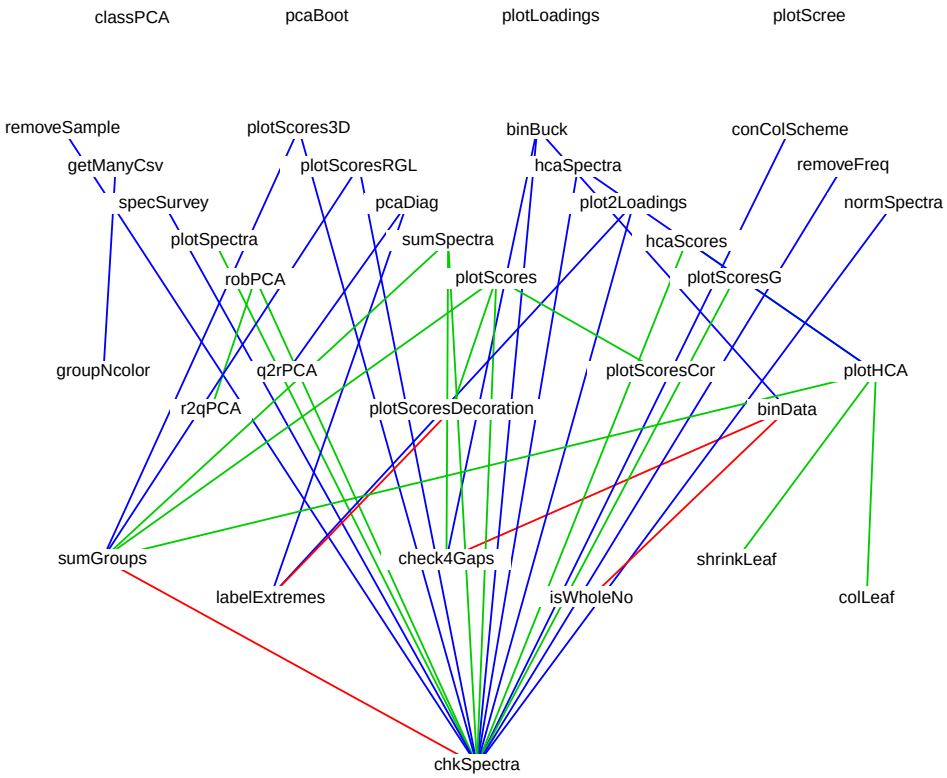
Example 15: Plotting One Loading vs. Another

```
> plot2Loadings(CuticleIR, class, title = "Cuticle IR Spectra",
+ loads = c(1, 2), tol = 0.002)
```



Example 16: Map of Functions in ChemoSpec

```
> foodweb(where = "package:ChemoSpec", charlim = 30, cex = 0.75, lwd = 1)
```



3 Technical Background

ChemoSpec is written entirely in R, there is no compiled code. Hence, it should be platform independent (please let me know if you discover otherwise). ChemoSpec uses S3 classes under the hood because frankly they were must faster to write. Converting to S4 would not be terribly difficult. For the pros and cons of classes and object-oriented programming in R, see the help archives (search my name for one thread and some really interesting replies from the big dogs). ChemoSpec employs several different graphics packages - the choice was one of practicality. I find it pretty quick to write in base graphics, but lattice and ggplot2 do certain things very well, so they were used where appropriate. Even rgl makes an appearance. In general, I tried to make all the graphics output look similar for consistency.

In the online documentation, there are sections which describe what other functions a given function calls (Calls ...), and what other functions are called by a given function (Called by ...). This information is more readily understood with a diagram like Example 16, generated by function foodweb in package mvbutils.

4 Acknowledgements

The development of ChemoSpec began while I was recently on sabbatical, and was aided greatly by an award of a Fisher Fellowship. These programs are coordinated by the Faculty Development Committee at DePauw, and I am grateful to them and those who created these programs. One of my student researchers, Kelly Summers, took the data included in CuticleIR in the summer of 2009 as part of a preliminary study. Prof. Dan Raftery at Purdue University provided an NMR data set (not included here) which was very helpful in troubleshooting functions.

5 The Competition

Several other packages have recently appeared which do some of the same tasks as ChemoSpec, and do other things as well. Spectrino is a GUI interface that runs only under the Windows OS. It runs as a separate program in communication with R. It is oriented mainly toward processing and organizing spectral data prior to statistical analysis. hyperspec is a very nice package that appeared while I was developing ChemoSpec, and it does many of the same things as ChemoSpec, and a few more. It is written using S4 classes which suggests that Claudia Beleites is a better computer scientist than me! Finally, the package Metabonomic provides a GUI interface to spectral processing such as baseline correction, as well as a range of exploratory and supervised statistical methods. Note: my comments here are based on the latest versions I have explored; newer versions may have considerably more features. Check 'em out for yourself!

References

[1] Bryan A. Hanson. *ChemoSpec: Exploratory Chemometrics for Spectroscopy*, 2009. R package version 1.30.

[2] P. Filzmoser K. Varmuza. *Introduction to Multivariate Statistical Analysis in Chemometrics*. CRC Press, 2009.