# Investigation of microplastics by FTIR-spectroscopy

FYS103

Spring 2022

## Aim

In the lecture on spectroscopy, you were introduced to infrared spectroscopy. You learned about vibrational modes in polar molecular bonds, and how these vibrational modes are utilized in infrared spectroscopy for chemical and structural analysis.You learned further that in any practical measurement, there are scattering and absorption signatures in infrared spectra and that it is important for the understanding and data analysis to retrieve the pure scattering features. During the spectroscopy tutorial you got acquainted with pre-processing of spectroscopic data, using the software Orange. During the course, you have also been introduced to principal component analysis (PCA). In the laboratory exercise at hand you will have the opportunity to use all the gained knowledge to investigate infrared spectra from two different microplastic types.

Initially, our aim for this exercise was to invite all of you to the BioSpec laboratories at RealTek to perform measurements yourself. Unfortunately, due to the ongoing corona situation, this is impossible at the moment. We have therefore performed the measurements, and you can follow the procedure in this video.

## 1 Introduction

During the past decade, microplastic has gained an increased interest and concern worldwide. While the long-term effects of microplastics deposits in the environment are not yet fully known, researchers are developing new methods for assessing the extent of microlitter prevalence. The aim is to find an alternative to time-consuming wet-chemical analyses. Promising methods for identification and quantification of microplastics is infrared microspectroscopy and infrared microspectroscopy imaging [1].

Infrared spectroscopy is a powerful tool for chemical and structural analysis of a variety of samples. The technique is used in different research questions, in fields ranging from biology to material sciences. Since the infrared spectrum represents a fingerprint of the sample, we often investigate groups of samples in spectroscopy. For example in biology we can consider spectra of healthy and diseased tissue, where the aim is to build a classifier which can be used in diagnostics. In our case, we have groups of plastic types. In classification tasks like these, the first analysis is often done by PCA. This is done to check if the spectra carry any information about the samples, such that we can group data naturally in for example healthy and diseased samples via the spectral fingerprint.

In spectroscopy, there is a variety of measurement techniques available, such as transmission measurements and reflection measurements. Different experimental setups allows for e.g. single measurements, infrared imaging and infrared tomography. Further, there are different ways to collect the infrared spectrum. The most common technique is Fourier-transform infrared spectroscopy (FTIR spectroscopy), where two parallel infrared beams are guided through the sample. By introducing a phase difference between the two beams by a movable mirror, so called interferograms are collected from which the spectrum can be calculated by Fourier-transform. The data set which is provided for this exercise is collected with an FTIR spectrometer, more precisely the instrument is a Bruker Vertex 70 FTIR spectrometer coupled with a Hyperion 3000 microscope, using a focal plane array (FPA) detector. The spectra are collected in transmission mode. Using an FPA detector, we collect infrared hyperspectral images, with one spectrum per pixel. With this technique, the whole

hyperspectral image is collected at once, which means that a background spectrum is first recorded in each pixel simultaneously, followed by the sample hyperspectral image with a full spectrum in each pixel. In order to facilitate your exercise, we have selected spectra of microplastic samples from the hyperspectral image. This means that you do not need to deal with an hyperspectral image cube, but a matrix of spectra with spectra as rows.

There are a lot of different interesting angles to take on this data set. You are free to choose the research question yourself, whether you choose to focus on the microplastics or more on the experimental method. However, remember to state clearly what the report will consider, and do not loose track of this later on. For finding more background information, we refer to literature [1].

# 2 Materials and methods

In a typical publications on FTIR and microplastics you would need to write a section called 'Materials and Methods'.

You will start this section about some general introduction on microplastics. To find out more about microplastics and identification of microplastics by FTIR, we refer to the publication which is uploaded to Canvas. You are free to search for more arcticles. On google scholar you can find all cited articles and all articles that cite this article. You will need to give a brief description of infrared spectroscopy as well. Depending on the research question you have defined in the introduction, make sure that the Materials and Method section contains the necessary background information.

As for the report on PCA, there is no need to go into details about the theory of PCA. You can keep this part to a minimum. But you need to mention with which software it was performed and refer to the software.

When you describe the method, it is important to provide information about the samples and the measurement technique. You do not need to go into details about how the instrument is used, but you should state what kind of equipment you have used. All parameters which were set during the measurement should be referred to such as spectral resolution and number of scans. Please remember that your spectra are obtained from an FPA imaging detector and that spectra referring to two microplastic samples have been selected from these images. You can describe this in your report. Please also have in mind that we used a transmission mode. This is the same mode as used in [1], while it is not exactly the same instrument producer. We used a Bruker Vertex 70 FTIR spectrometer coupled with a Hyperion 3000 microscope, using a focal plane array (FPA) detector.

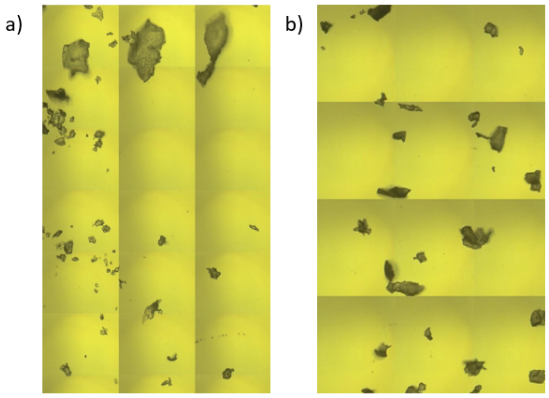
The samples were produced by cooling down plastic pellets with liquid nitrogen, and subsequently grinding them in a ball mill. These pellets are commonly used in industry to manufacture different plastic objects. This means for our study the samples where produced artificially, they were not collected as microplastic from the environment. The reason for producing microplastic particles our self instead of collecting them from the environment is due to issues with labeling the data. When you have a mixture of unknown particles, it is challenging to label them one by one. We need these labels to study whether different plastic types can be distinguished by FTIR spectroscopy or not.

The instrument used for collecting the infrared spectra are presented in this video. The number of scans was set to 32 for both the background and sample, and the resolution was set to 8 $cm^{-1}$. Since the interferogram is Fourier transformed to obtain the spectra, spectral resolution is not the same as the spacing of the wavenumber readings. Please have in mind that you do not need to explain all the single steps performed when running the spectrometer. However, you need to report parameters chosen etc. Please check the literature to understand what is reported [1].

# 3 How to proceed

## 3.1 Uploading and inspecting spectral data

The data set used in this exercise can be found on canvas, under the filename *microplastics.xlsx*. Upload this to Quasar, and inspect the data using the widgets data table and spectra. The sample names start with either *PET* or *PLA*, referring to polyethylene terephthalate and polylactic acid, respectively. To make the plastic type a meta value, use the widget **create class**. When the plastic type is available as a meta attribute, it is possible to colour the spectra according to the groups




*Figur 1: Visible images of the microplastic samples, a) polylactic acid (PLA) and b) polyethylene terephthalate (PET).*

and later scores in PCA plots. Do you see any differences between the groups when inspecting the raw spectral data?

## 3.2 Pre-processing

As you have seen in the lecture on spectroscopy, there are different ways to pre-process spectral data. The aim is to remove physical effects and to enhance the band position and shapes. Play with the **preprocess spectra** widget, using the Savitzky-Golay filter and EMSC. Use the widget **average spectra** to inspect the group averages. What do you see? When you try different windows in the Savitzky-Golay, you will see that large windows have a smoothing effect. Does it affect the results? Remember to state the parameters used when reporting the results.

## 3.3 PCA analysis

After pre-processing, perform PCA on the data. Unlike in the laboratory exercise on PCA which you have already done, we usually do not normalize the data when we we perform PCA on spectroscopic data. This is due to the fact that all variables are related to the same measure, they are not independent and not on a different scale. From the PCA widget you can visualize the score plot (using a scatter plot), or the components. To view the components, connect a spectra widget to the PCA widget, and make sure the connection sends “components” to “data”.

If you want to use only specific spectral regions for the PCA, select this with the **select column** widget.

Questions to have in mind when working with this data:

- What kind of pre-processing works best for this data?
- Perform EMSC with and without Savtizky-Golay filtering first.
- Is it easy to separate the two plastic groups with PCA using only the raw spectra? Why or why not? If this is the case, why do we need to pre-process the data first?
- When you investigate the spectra with and without pre-processing: how do the loadings change? Both scattering and chemical absorption may be different for different (plastic) particles. Therefore PCA may find both scattering features and chemical features to display clusters. Check if you obtain nice grouping because of scattering or because of chemical absorption. The trick is to check if there are scattering features in the loadings or only pure absorption features. You can compare the loadings for raw data and data that is pre-processed with EMSC (without Savitzky-Golay).
- Another parameter to investigate is the window size for the Savitzky-Golay. What happens if you choose a very large window size for the Savitzky-Golay filter (for example 71)?

- Do you find any outliers?
- You are also free to split the spectra into different spectral regions as you did for the Listeria data set. Does the use of a specific range improve results? Which spectral regions are best suited for separation of polyethylene terephthalate and polylactic acid?

# 4 Discussions and error analysis

Depending on what you have set up as a research question, discuss your findings considering this. In the discussion section, we usually compare our results with results in the literature. Use the papers on Canvas, or other sources, and see if you can draw any parallels to previously published work. Do you find any sources which indicates which absorbance bands you would expect to find in the two plastic types?

For doing a typical error analysis for this type of data, you would need to have methods such as multivariate regression, artificial neural networks etc. Since we do not have these methods at hand, you need to describe the 'error' qualitatively. How well are the groups defined in the PCA plot? Do you find any outliers in the PCA analysis? How do the spectra look? Is the baseline standardized? Is there a lot of scattering in these data? Can you say something about the signal to noise ratio? Is that consistent within the data set? From the instruction video you can get an overview of how the instrument is used, and possible pitfalls. Key factors are: adjusting the condenser position, stability of the measurement atmosphere and number of scans that are averaged.

# Referanser

[1] Márta Simon, Nikki van Alst og Jes Vollertsen. "Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging". I: *Water research* 142 (2018), s. 1–9.