Manual SpectraByte™ V3.5

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MantiSpectra

1. The Spectral package

The spectral package includes:

- SpectraPod™ (see following sections for different types)
- A white reference
- Mini-USB cable
- A cuvette holder (optional)
- Software

1.1 SpectraPod™ – Reflection

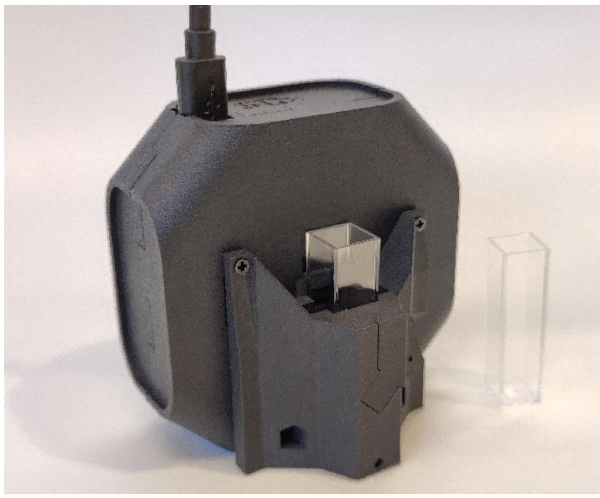
- Measure the reflection of solids by placing them on top of the SpectraPod™ – covering the optical window to reduce the influence of the environmental light.
- A cuvette holder can be attached to measure the reflection of liquids/powders.


1.2 SpectraPod™ – Fiber

- This SpectraPod™ type allows users to measure the output of a SMA905 optical fiber by connecting it to the SMA connector on the SpectraPod™.
- For maximum stability, use the Velcro-strap to hold the fiber in place and reduce the possible movement in the connector.


1.3 SpectraPod™ – Transmission

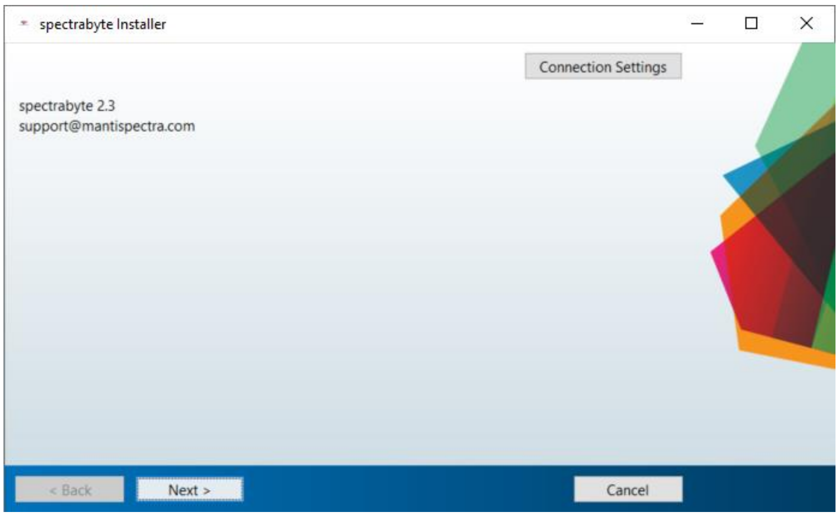
- The lamp integrated in the cuvette holder is used to measure the transmission through liquids.
- This SpectraPod™ can measure in transmission only, the lamp used in the reflection mode is not present.
- The cuvette attachment cannot be removed. This will damage the wires powering the lamp.



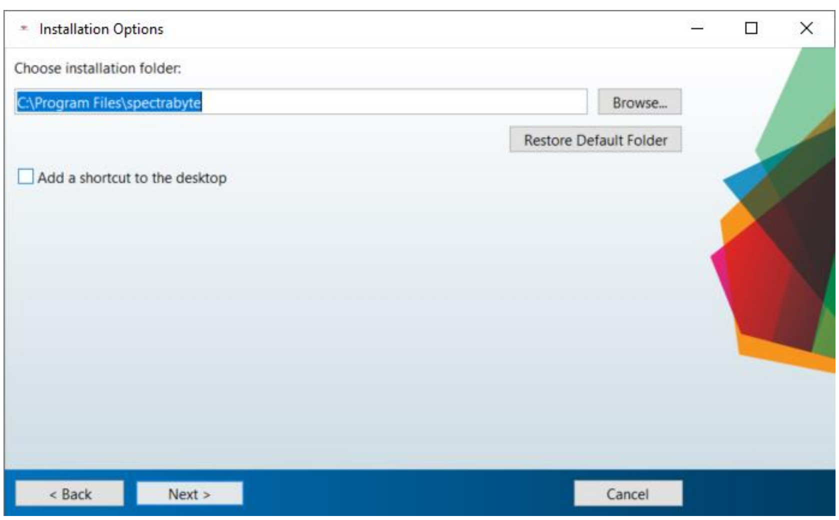
2. SpectraByte™ software

2.1 Installing the SpectraByte™ software

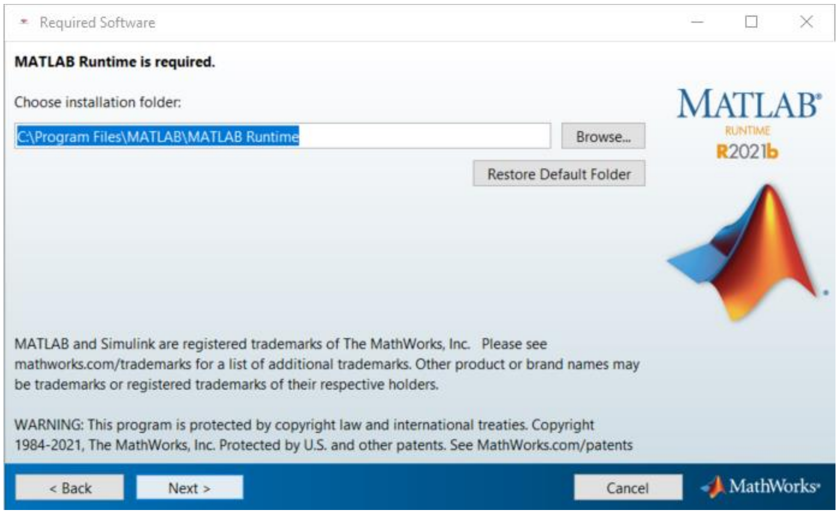
To install the SpectraByte™ software, open 'Installer_SpectraByte_v32.exe' (this may take a few minutes). When the screen shown below opens, click 'Next'.



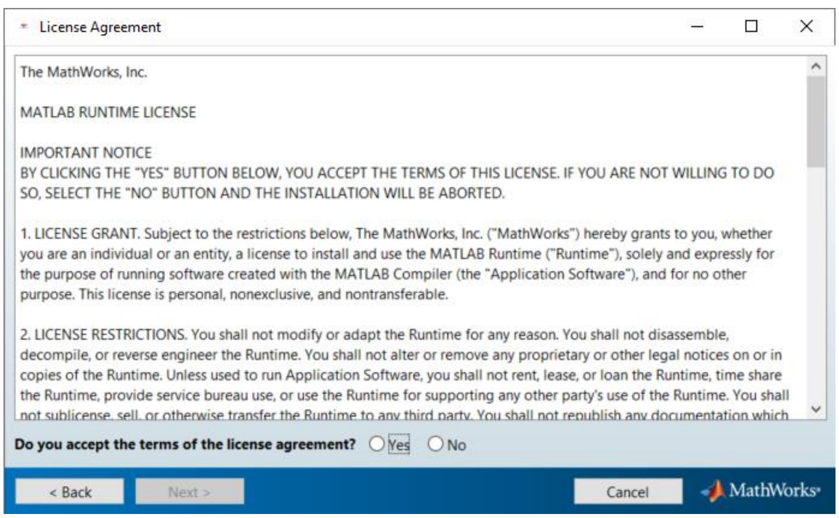
Select the folder where you want to save the SpectraByte™ software using the 'Browse' button and click 'Next'.



Select the folder where you want to install MATLAB Runtime and click 'Next'. This software is required to run the SpectraByte™ software. Please note that you do not need to have a MATLAB license for this.



Please accept the MATLAB Runtime License agreement and click 'Next'.



Begin the installation by clicking 'Install'. The installation may take a few minutes.

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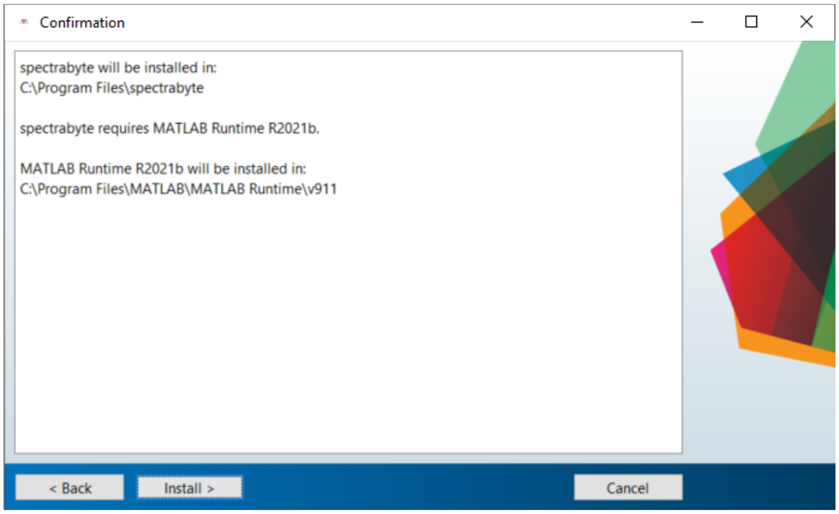
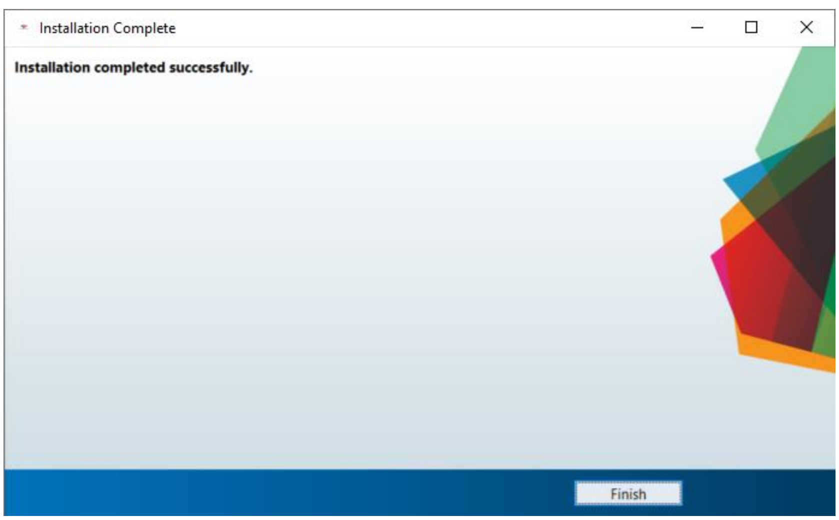


After the installation is finished, the following screen will appear. Click 'Finish', to close the installation wizard. The SpectraByte™ app is now installed on your PC.



The app can be opened via 'spectrabyte.exe', which can be accessed via: 1) the selected folder e.g. *C:\Program Files\MantiSpectra BV\spectrabyte\application*; 2) the search bar; or 3) the desktop (Only when 'Add a shortcut to the desktop' was selected).

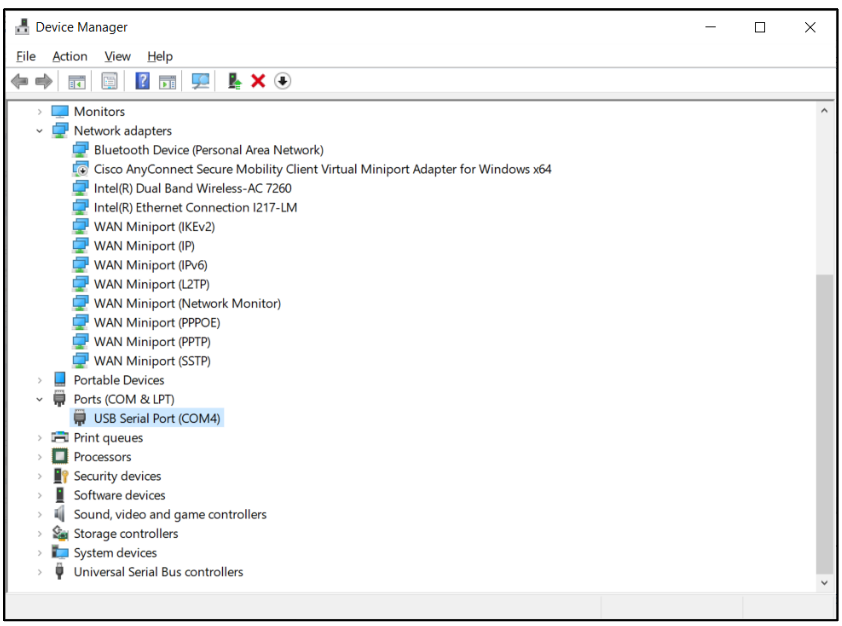
3. Driver Installation

This chapter explains how to install the drivers required to connect to your SpectraPod™ with the SpectraByte™ software.

3.1 Identify COM number

Connect the SpectraPod™ via the provided mini-USB cable to your PC. In order to setup a communication channel between your SpectraPod™ and SpectraByte™, you need to identify the COM port of your SpectraPod™. Follow the steps below:

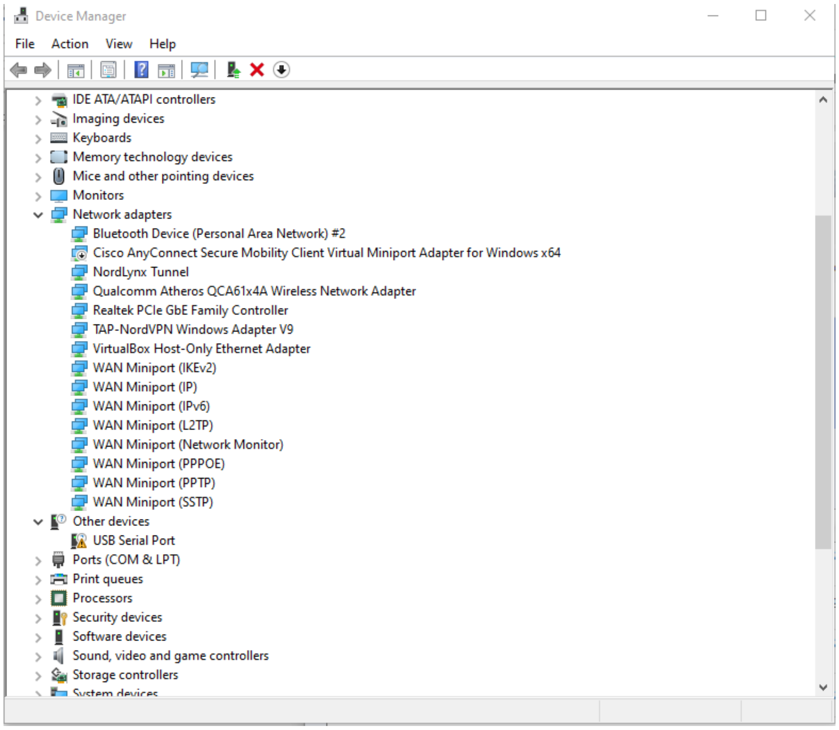
Open 'Device manager' on your PC (this can be done via the search bar in Windows or by right clicking on Start to open the 'Power user menu' and select 'Device manager'. → Go to 'Ports COM & LPT' and identify the COM number of SpectraPod™. For example *COM4*, as shown in the image below. Remember the COM number.



In case the SpectraPod™ is not automatically recognized as a COM port and not visible in the 'Ports (Com & LPT)' list, please follow the steps in **Section 3.2** Setup of the COM port. Otherwise proceed to **Chapter How** to use SpectraByte™.

3.2 Setup of the COM port

Open the Device Manager on your pc: the SpectraPod™ will appear in the 'other devices' category. See figure below, where the SpectraPod™ is shown as 'USB serial Port'.



Download the TDI driver (Windows 10 version 1803 and Later Servicing Drivers, Windows 10 Version 1803 and Later Upgrade & Servicing Drivers) from here:

https://www.catalog.update.microsoft.com/Search.aspx?q=usb+serial+converter+ftdi+10

Save the driver in an easily accessible location on your pc.

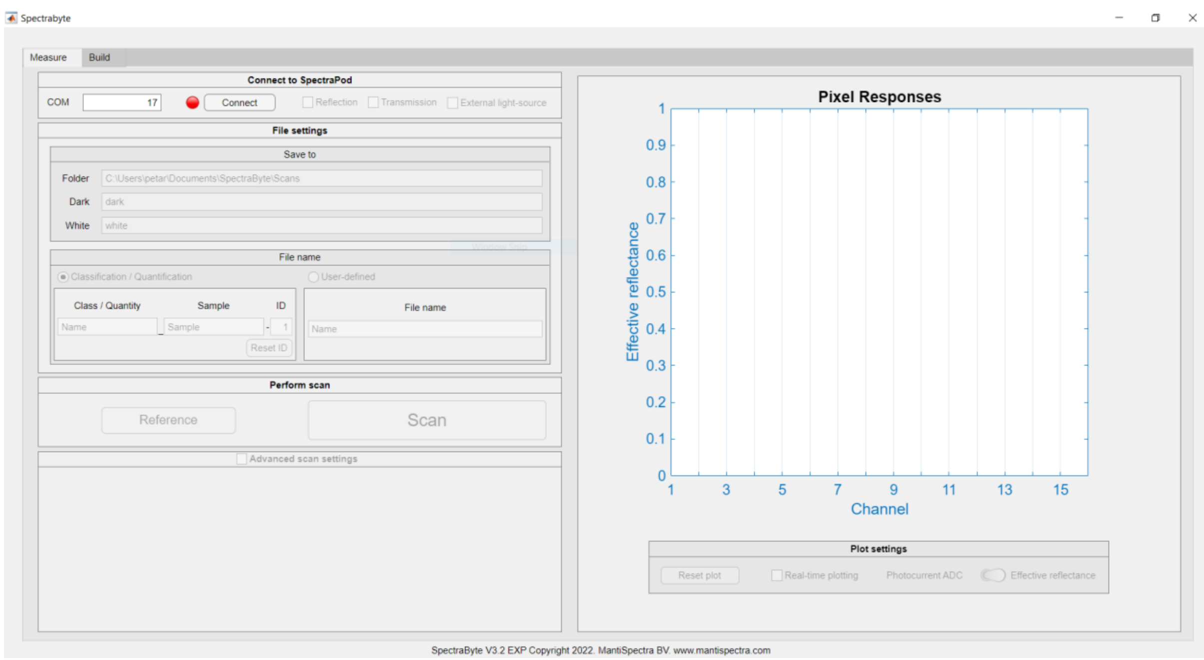
Right-click in the device manager on the 'USB Serial Port' → 'Update Driver' → 'Browse my computer for drivers' → Browse and select the directory where you have saved the driver.

After these steps, your SpectraPod™ device should be recognized as a COM port and you can follow the steps as explained in **Section 3.1** Identify COM number.

4. How to use SpectraByte™

After installing SpectraByte™ and setting up the required drivers, open the SpectraByte™ application. The app can be opened via 'spectrabyte.exe', which can be accessed via: 1) the selected folder e.g. *C:\Program Files\MantiSpectra BV\spectrabyte\application*; 2) the search bar; or 3) the desktop (only when 'Add a shortcut to the desktop' was selected in the installation wizard).

Fill in the COM text-field the port-number according to the value found in the 'Device Manager' (see section 3.1).

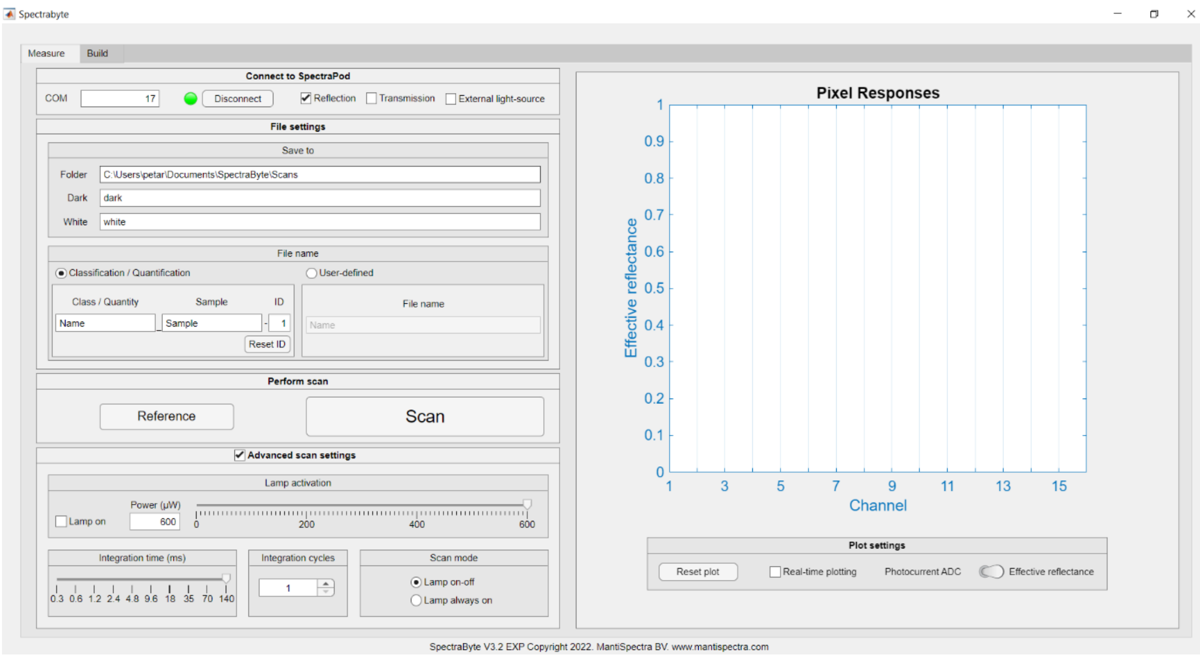


Click 'Connect'. If the connection is successful, the button will appear green and the button text changes to "disconnect". If the connected device contains an internal lamp, it will flash for 1 second.

Choose the measuring method you will use – Reflection, Transmission or with an external light-source – by selecting the respective checkbox.

4.1 Software overview

A quick overview is given here of the SpectraByte™ software functionalities.



In the 'File Settings', the file-locations and file names can be selected.

- Folder: All files will be saved in the folder specified in this text-field. The standard folder is: C:\Users\<username>\Documents\SpectraByte\Scans, where <username> is the active user on the PC. If this folder does not exist, it will be created automatically. A user can specify any other folder path here, if required.
- Dark: The filename for the dark-reference measurement (see **Section Reference measurement**).
- White: The filename for the white-reference measurement (see **Section Reference measurement**).
- File name: There are two options to choose the required file name i.e., 'Classification/ Quantification', which has a predetermined format with an incrementing 'ID', allowing for easier file-management and data-exporting; and 'User-defined', which gives the user full flexibility to choose a filename.

The 'Perform scan' block contains two buttons: 'Reference' and 'Scan', whose functions are explained in detail in **Section Reference** measurement and **Section Sample** measurements respectively.

Checking the 'Advanced scan settings' reveals additional settings to customize your measurement routine.

- Lamp activation: the lamp Power can be changed using the numeric field or slider. The shown illumination power in μW is an estimation and can deviate from the actual power. We recommend using the maximum power, unless required differently for your specific application. The 'Lamp on' checkbox will turn the lamp on so the user can check the illumination visually. While the lamp is turned on, no reference or scan can be carried out, so this box must be unchecked in order to proceed.
- Integration time: The integration time of the sensor can be set between approximately 0.3 and 140 ms per channel, using the integration time-slider. We recommend the highest integration time for the best results, unless your specific application requires differently.
- Integration cycles: This value indicates the number of scans performed in a single measurement. 1 cycle is sufficient for most applications; however, the number of cycles can be increased to reduce the effect of external fluctuations.
- Scan mode: In this panel you can choose between: 'Lamp on/off' and 'Lamp always on'. 'Lamp on/off' turns the lamp on during a scan only and keeps it turned off otherwise. 'Lamp always on' leaves the lamp continuously and indefinitely on.

By default, the maximum lamp power, the longest integration time, 1 integration cycle and 'Lamp on/off' are selected. Unchecking the 'Advanced scan settings' returns all settings to the default settings.

The 'Plot settings' on the bottom right of the screen control the data visualization in the 'Pixel responses' figure above. Additional details can be found in **Section Sample** measurements.

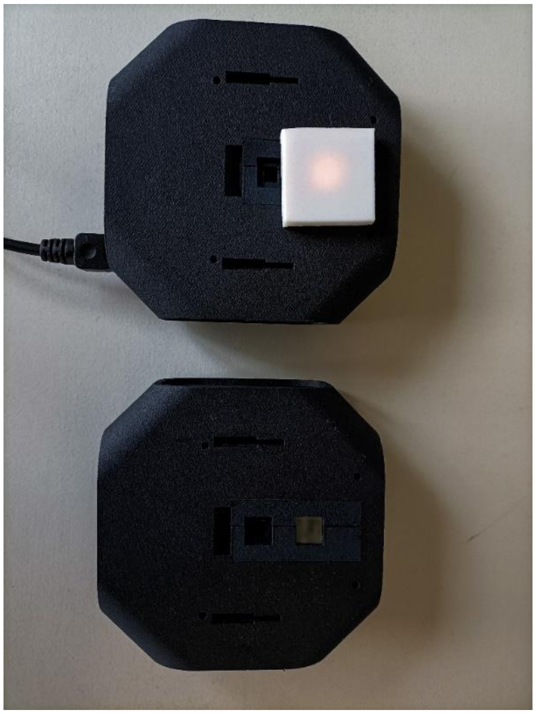
- Reset plot: Clears the figure of all current graphs.
- Real-time plotting: This will keep plotting new measurements in real-time without saving anything. While real-time plotting is turned on, all the other functionalities are turned off to allow the device to keep transferring the scan-data.
- Photocurrent ADC & Effective reflectance/transmittance: This toggles the visualization of the measurement results between the Photocurrent ADC or Effective reflectance/transmittance.

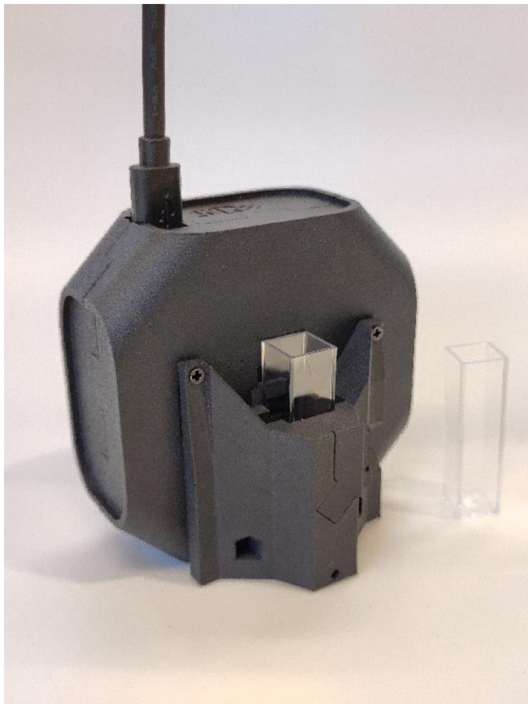
4.2 Reference measurement

Before starting any measurements, we advise to connect the SpectraPod™ to your PC and wait 15 minutes to stabilize the system.

A reference is required at the start of each measurement session. The reference requires a dark- and a white-reference measurement, which are used for correcting the environmental light and normalizing to a known reflection/transmission reference. It is good practice to repeat the reference measurement every 2-3 hours that the device is used; or when the environment – such as temperature, humidity or surrounding light – has changed. The reference method for a reflection-SpectraPod™ differs from the method for a transmission- SpectraPod™.

- Reflection: To take the reference measurements the reflectance standard should be placed on top of the SpectraPod™ in such a way that it covers the full measurement window (see left picture below). If a cuvette holder is used, it should be removed to take the reference.
- Transmission: Place an empty cuvette inside the cuvette holder (see right image below). The transmission through the empty cuvette is used as the white reference.





The reference data will be saved in the folder that is specified in the 'Folder' text-field with the filenames from the 'Dark' and 'White' text-fields (see **Section Software** overview). Click the 'Reference' button and wait until the program notifies you that it completed the reference.

- The dark-file contains the raw ADC values measured with the internal lamp turned off and without any incoming environmental light (since it is shielded by the reflectance standard). The values represent the electrical current that flows through a detector even when no light is falling onto the sensors.

- The white-file contains the raw ADC values measured with the internal lamp turned on which is diffusely reflected by the reflectance standard (in reflection mode) or transmitted through an empty cuvette (in transmission mode). This measurement is used to calculate the 'Effective reflectance/transmittance'.

Finally, it is advised to use the same settings (lamp power, integration time and number of integration cycles) for the reference measurement, as in the actual measurements.

4.3 Sample measurements

After taking the reference, your device is ready to measure your samples. First of all, there are two options to choose a preferred filename:

- Classification/Quantification: A predetermined file-naming format which contains 3 text-fields. 1) Class/Quantity, to enter the name or quantity of the material you want to classify or quantify (for example PP, HDPE, PS, etc for plastic classification or 0, 5, 10, etc for alcohol percentage quantification). 2) Sample: keep track of the sample number or name, since most classes consist of multiple samples to capture all the possible variations (such as different colors, shapes, thicknesses, etc) in your model. 3) ID: it is good practice to measure all samples multiple times, but especially inhomogeneous samples should be measured from different sides to include this information into the model. The ID-number increments after each measurement to keep track of the measurements. The value can be set back to 1 using the Reset ID button.
  Additionally, using this format assists with automatic data clustering for easier model creations in future steps (**see Section 4.4**).
- User-defined: To give our users full flexibility, this option allows you to specify any filename you require.

With the chosen scan-settings (default ones or personally adjusted), reference taken and selected filename, the device is ready to scan your samples. In reflection mode, cover the optical window as good as possible with the sample to reduce the influence of the environmental light; place the cuvette containing your sample in the cuvette holder in transmission mode; or place the fiber-probe in the desired location using the fiber mode, and press 'Scan'.

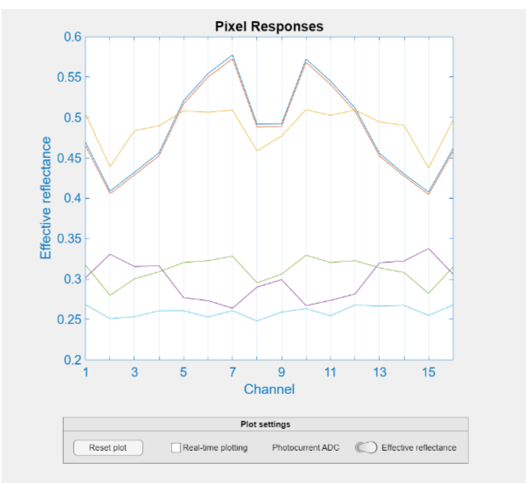
For each scan, two files are saved with a .dat extension.

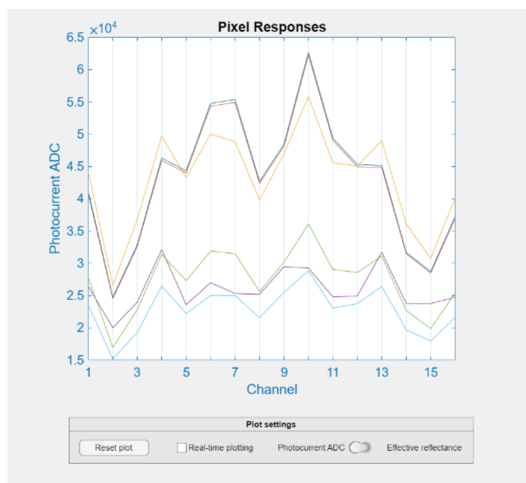
- *Filename-raw.dat*: contains the raw data measured in all acquisition cycles. The 16 rows represent the 16 channels and there is a column for each cycle. The value set in 'Integration cycles' determines the number of columns e.g. when the value is set to 1, a single column is generated.

- *Filename.dat*: always contains three columns.
    - Column 1: The Photocurrent ADC values – dark-corrected and averaged over all cycles.
    - Column 2: The standard deviation of the cycles per Spectral-Channel i.e., the standard deviation of each row from the raw.dat file. In case the number of 'Integration cycles' is set to 1 (by default), the standard deviation is zero for all Spectral-Channels.
    - Column 3: The effective reflectance/transmittance i.e., the dark-corrected average values (column 1) normalized to the white reference (also dark-corrected).

The acquired scans are plotted in the 'Pixels responses' figure. By default, the plotted values are the Effective reflectance/transmittance as function of the Spectral-Channel number. The Effective reflectance/transmittance represents the dark-corrected photocurrent ADC values, normalized to the White reference (also dark-corrected). The figure can be changed to show the dark-corrected Photocurrent ADC as function of Spectral-Channel number using the 'toggle switch' in the bottom right.

The images below show several example measurements which indicate the differences between the two plotting mechanisms. The 'Effective reflectance' gives a better insight in the minimal spectral differences between the various samples compared to the 'Photocurrent ADC', since the effective reflectance/transmittance already contains a first normalization step.



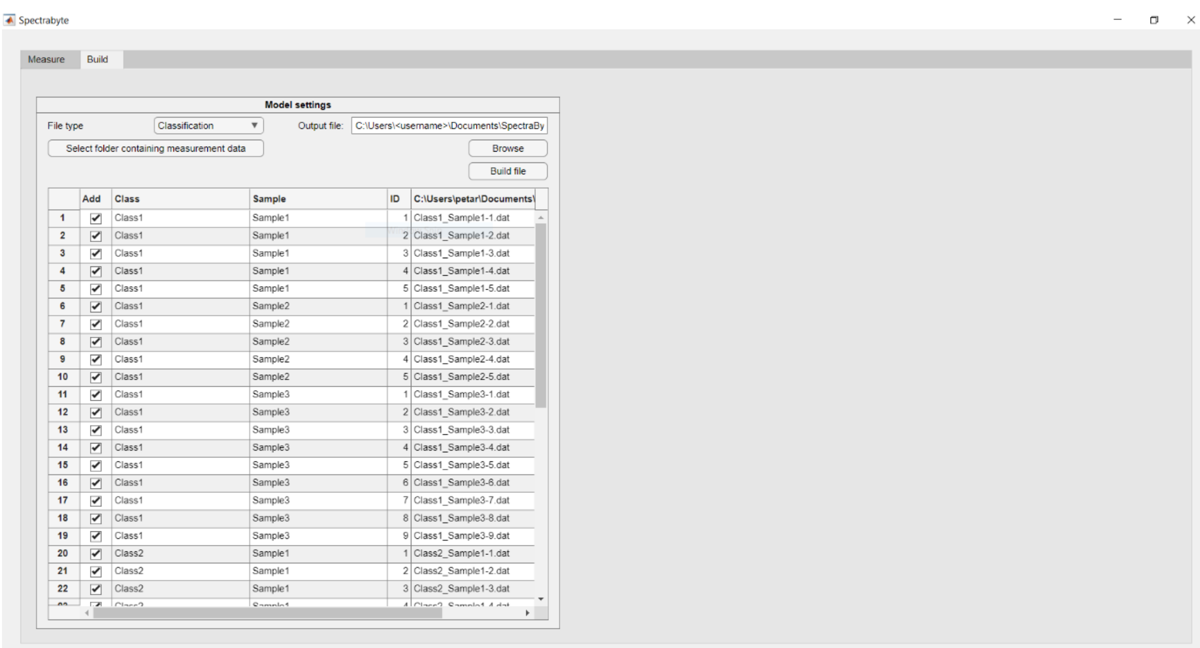


4.4 Building a prediction model

The measurement data can be used to build a predictive model using chemometrics. We recommend trying a few standard chemometrics algorithms for classification, such as: principal components discriminant analysis, partial least squares discriminant analysis, support vector machine classification, nearest neighbor classification, and random forest classification. Of course, other methods are also possible.

In addition, various normalization methods could increase the predictive accuracy of a model. There are a broad range of normalizations found in literature – a common method is sum-normalization: for a single measurement, divide the value of each individual Spectral-Channel by the sum of all 16 Spectral-Channels. Other normalization methods could also be tried, depending on your application.

To assist users in managing their relevant data, SpectraByte™ has an additional tab called 'build', to build and export a single .csv file containing all measurements required for a model.



- In the 'File-type' drop-down menu, you specify the file-naming system you used during your measurements i.e., 'Classification', 'Quantification' or 'User-defined'. The options 'Classification' and 'Quantification' will fill in the correct information in the table automatically.

- 'Select folder containing measurement data' opens a file-explorer window where you should navigate to the folder that contains all the data that you wish to include in your model. The software will then automatically detect the relevant files and import the information into a table. In the example above, a classification is built where 'Class1' contains 3 samples and 'Class2' contains 2 samples; all samples are measured 4 times and all measurements should be included in the exported CSV file, since all the checkboxes in the first column are selected.
- Select the name and location of your CSV file either by typing the full path directly in the 'Output-file' textbox, or by pressing the 'Browse' button which will open a file-explorer window.
- Finally, press the 'Build file' button to export the CSV file.

The exported CSV file has a specific format as shown below: the 'sample' corresponds to the filename of each measurement and the 'referenceValue' corresponds to the class or quantity given by the user. 'WC' and 'DC' behind the pixel numbers indicate the white-corrected (effective reflectance/transmittance) and dark-corrected values respectively.

	A	B	C	D	E	F	G	H	I	J	K
1	sample	referenceValue	pixel_1_WC	pixel_2_WC	pixel_3_WC	pixel_4_WC	pixel_5_WC	pixel_6_WC	pixel_7_WC	pixel_8_WC	pixel_9_WC
2	Class1-Sample1-1	Class1	0.072520095	0.077258407	0.074988793	0.074336061	0.0705983	0.070890561	0.069795741	0.072272027	0.069953842
3	Class1-Sample1-2	Class1	0.072508412	0.077150834	0.075381038	0.074712422	0.070417321	0.070369078	0.070383477	0.072286034	0.070267118
4	Class1-Sample1-3	Class1	0.072418842	0.077155956	0.075086854	0.074625569	0.070639432	0.070496859	0.070292419	0.072075921	0.069990502
5	Class1-Sample1-4	Class1	0.072239703	0.077171324	0.07524095	0.074728506	0.070326832	0.070472684	0.069692266	0.071967362	0.070290447
6	Class1-Sample1-5	Class1	0.072504517	0.076551495	0.075086854	0.074583752	0.070256908	0.070921643	0.069410815	0.072149461	0.069893853
7	Class1-Sample2-1	Class1	0.072360427	0.076915196	0.074853375	0.074529067	0.070092382	0.07027238	0.069762629	0.071988374	0.070140474
8	Class1-Sample2-3	Class1	0.07243442	0.076741029	0.074293026	0.074352145	0.070627093	0.070697164	0.069994412	0.071662698	0.069870524
9	Class1-Sample2-4	Class1	0.07214624	0.07682299	0.074867384	0.07429746	0.070635319	0.070320729	0.069518429	0.071655694	0.070493743
10	Class1-Sample2-5	Class1	0.072169606	0.077027892	0.074806679	0.07416879	0.070063589	0.070472684	0.069568097	0.071582154	0.069990502
11	Class1-Sample3-1	Class1	0.07237211	0.077309633	0.074633905	0.074007952	0.070170532	0.070600465	0.070139277	0.071932343	0.069570579
12	Class1-Sample3-2	Class1	0.072298118	0.076643701	0.07424633	0.074368229	0.070680564	0.070476138	0.070511786	0.071676705	0.069533919
13	Class1-Sample3-3	Class1	0.072122874	0.076064852	0.074615227	0.073921099	0.070664111	0.070641907	0.07000269	0.071837792	0.069540584
14	Class1-Sample3-4	Class1	0.072640819	0.076838358	0.074703949	0.074284593	0.070947919	0.070534848	0.069373564	0.071582154	0.070300445
15	Class1-Sample3-5	Class1	0.072446103	0.076756397	0.074717957	0.073995085	0.070388529	0.070351811	0.069845409	0.07196386	0.070117145

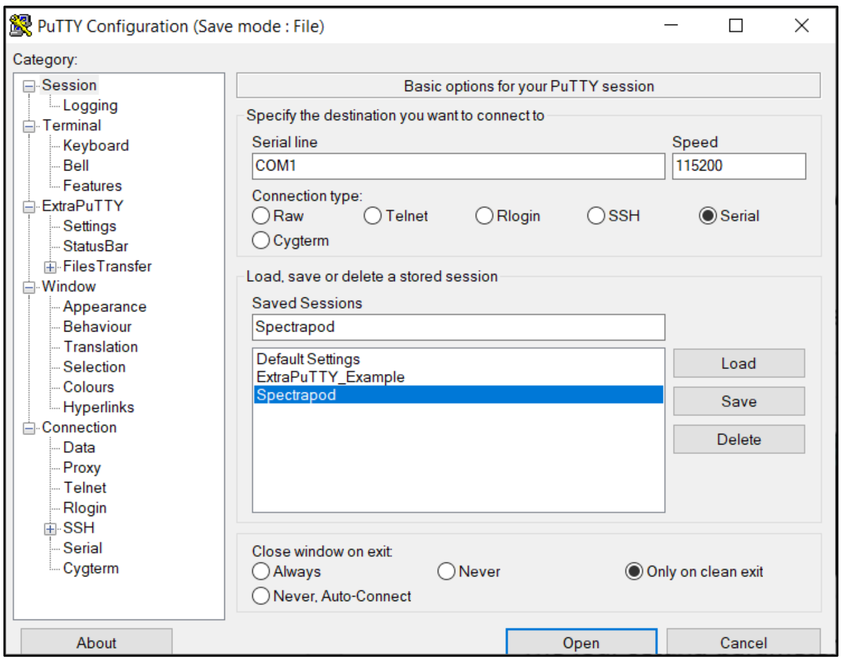
5. Measurements via Terminal

5.1 Set up the terminal

Please download and install ExtraPutty to interrogate the SpectraPod™ via a command line:

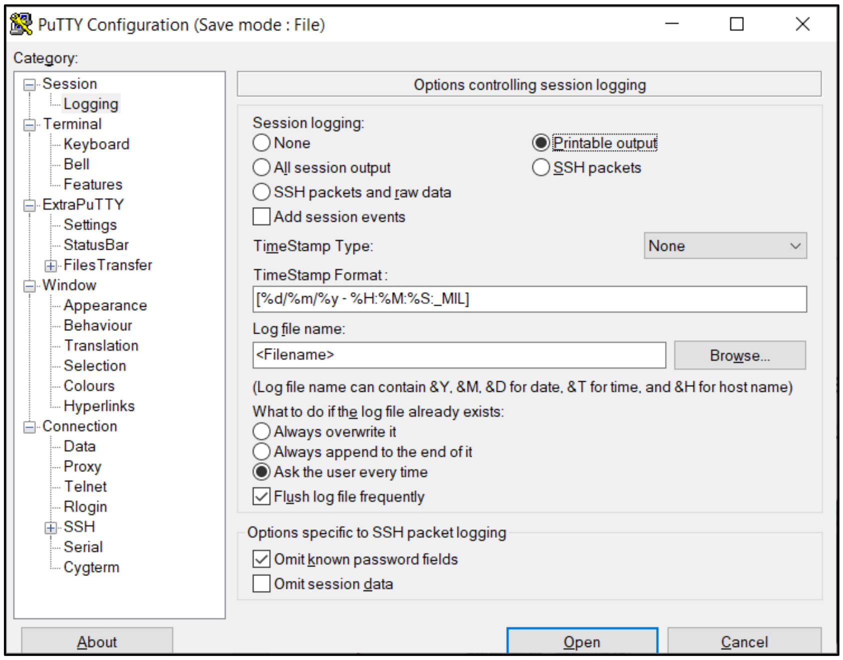
http://www.extraputty.com/download.php (installer version 0.30)

Configure ExtraPutty with the following settings: 'Serial line' the COM number assigned to SpectraPod™, 'Speed' = 115200. Save the session by specifying a name in the 'Saved Sessions' field and click 'Save'. See the figure below.



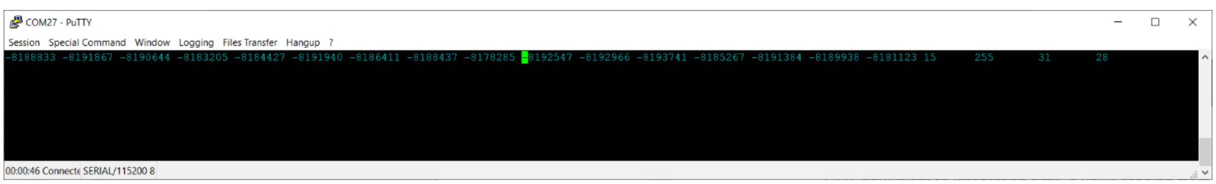
Open the 'Logging' tab (top left), and select the following:

- 'Session Logging': 'Printable Output'.
- 'Timestamp type': 'None'.
- Specify the preferred file location in the 'Log File name' field using the 'Browse' button.



5.2 Read-out via terminal

To open the session click 'Open'. Once the session is opened, the raw data from the 24bit ADC for the 16 channels array will be displayed, followed by four setting parameters. See image below.



The four setting parameters indicate:

- OSR, equivalent to integration time: a value ranging from 1 (high speed) to 15 (low speed).
- Internal Lamp power setting: 0 = not activated, 255 = full lamp power.
- Humidity sensor.
- Temperature sensor.

5.3 Change internal lamp power and integration time

To access the terminal to change the internal lamp power or the integration time: press 'm' on the terminal window. This command will stop the read-out and enter the settings mode.

To change the internal lamp power: write *XXv*, where *XX* is a hexadecimal value ranging from 0 to 255. Please note that the maximum power is already reached for a value of 150 (approximately 600 μW).

- To set the maximum power write: *ffv*. See image below.
- To turn the lamp off write: *0v*.

To change the integration time: write Yo, where Y is a hexadecimal value ranging from 1 to 15, excluding 10 to 14. 15 represents 512 ms and 1 represents 1 ms. The integration times can be set to one of the following values: 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 ms, using the set values 1, 2, 3, 4, 5, 6, 7, 8, 9 and 15 respectively.

- To set the maximum integration time write: *fo*.
- To set the minimum integration time write: *1o*.

Press 'm' to return to the reading mode.